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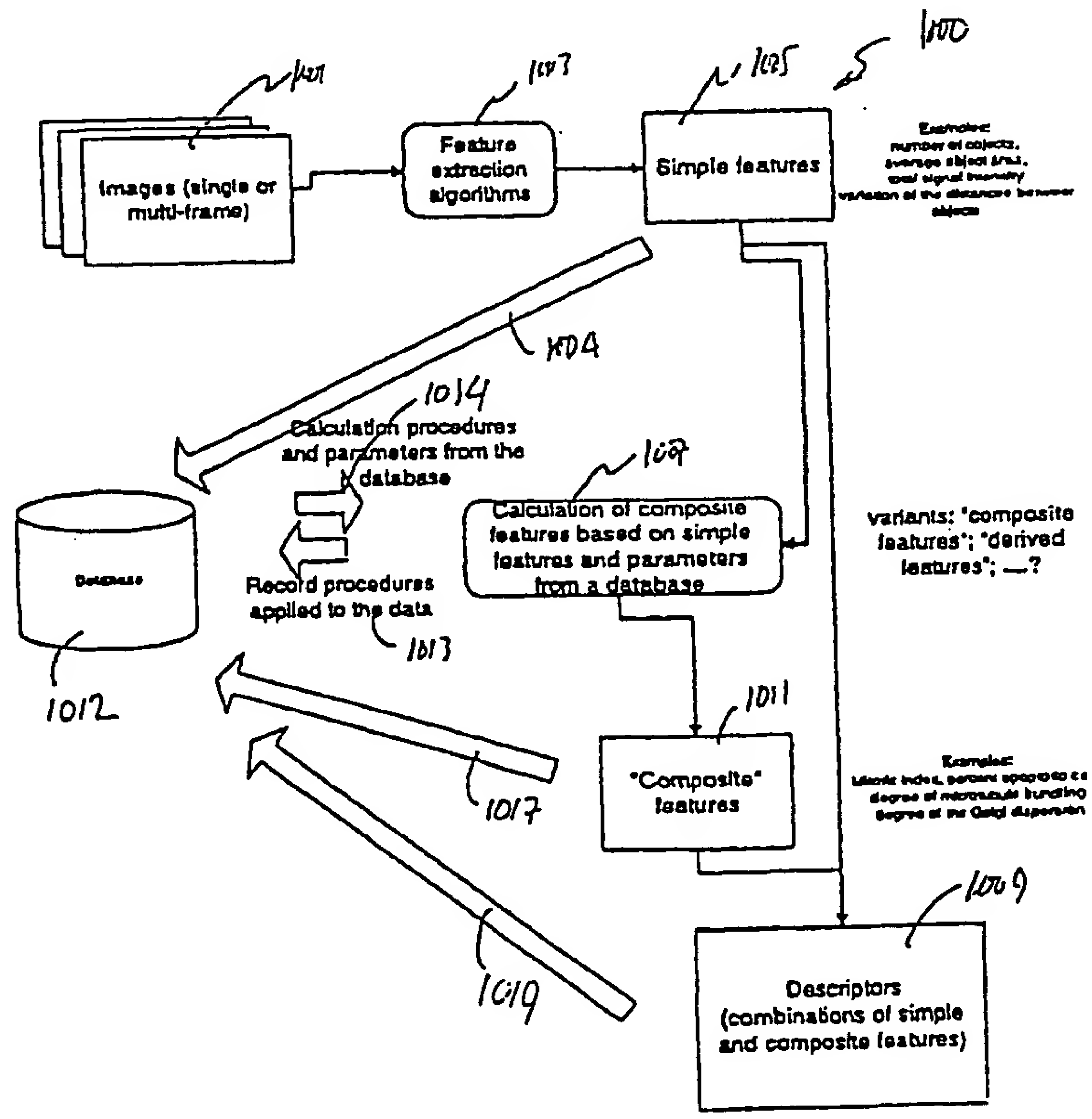
(57) Abstract

Techniques for using information technology in therapeutics or drug discovery. In an exemplary embodiment, techniques for determining information about the properties of substances based upon information about structure of living or non-living cells exposed to substances are provided. A method according to the present invention enables researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database. The present invention further teaches a system for acquiring knowledge from cellular information. The system has a database 1012 comprising a database management module ("DBMS"). The system also has a variety of modules, including a population module coupled to the DBMS for categorizing and storing a plurality of features (e.g., cell size, distance between cells, cell population, cell type) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.



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PATENT APPLICATION
METHOD AND APPARATUS FOR PREDICTIVE CELLULAR BIOINFORMATICS

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BACKGROUND OF THE INVENTION

The present invention provides techniques for information management using a database platform. More particularly, the present invention provides a system including computer code that couples to a database device. The system provides for image capturing of living, dead, or fixed cells or cell fractions used to identify information about substances used on the cells or information about the cells themselves. Accordingly, the present invention can enable researchers and scientists to identify promising candidates in the search for new and better medicines, for example, in drug discovery and development. The principles enumerated herein may, with equal facility, be applied to other applications, including but not limited to use in environmental applications such as determining chemical toxicities and other non-pharmaceutical toxicology uses.

For a long time, researchers in the pharmaceutical field have sought for better ways of searching for substances possessing properties that make them suitable as medicines. In the early days, researchers generally relied upon extracts from plants, dyes, and microbiological extracts for such substances. Examples of such substances include the pain reliever aspirin, the anti-cancer drug paclitaxel (brand name Taxol™), and the heart medication called digoxin. The number of useful medicines has generally been limited.

Purified substances having desirable bio-active properties are also often difficult to discover. Advances in traditional organic chemistry and more recently the rapid chemical synthesis methods often referred to as combinatorial chemistry have increased the number of compounds that researchers test for biological activity. Originally, substances were often initially tested on animals or humans to determine their biological activity. While results from such tests may identify a good drug candidate, they are often time consuming and costly, thus a limited number of substances can be tested. Therefore, pharmaceutical companies have turned to testing their ever-increasing libraries of substances against isolated proteins (drug targets) in biochemical assays that can be carried out at high throughput and low cost. It should be noted that the substances need to be tested in numerous protein tests, each customized for a particular drug target. Therefore, although each protein test may be run at a high-throughput, the design of multiple protein tests can be time-consuming. Substances deemed promising based on results from the protein tests are then tested in lower throughput cellular and animal tests.

There have been some attempts to use image acquisition techniques to screen a large number of substances based upon biological cell information. One such attempt is described in International Application No. WO 98/38490 in the names of Dunlay, et al. Dunlay et al. generally describes a conventional image acquisition system. This conventional system collects and saves images based on certain criteria that are predefined, not on a fixed area of an imaging surface. Additionally, the conventional system has poor lighting design, which makes image processing for multiple cells difficult. Furthermore, the conventional system is not designed for capturing, populating and utilizing a large database design. The conventional system is designed for customized cellular assays, not as a tool for generation of a cellular informatics database. Without such database capabilities the conventional system cannot be used for screening, analyzing, and comparing large quantities of cells from multiple experiments on multiple days in a predictive, efficient and cost effective manner.

What is needed is a rapid assay to assess the activity of compounds against multiple drug targets simultaneously in a cellular context. What is also needed are techniques for finding the effects of substances on cell function based upon searching and analyzing cellular information.

SUMMARY OF THE INVENTION

According to at least one embodiment of the present invention, techniques for determining information about effects of potential substances on cells are provided. In another exemplary embodiment, the present invention provides a novel system including hardware, computer codes, user interfaces, and a database for acquiring, storing and retrieving cellular and substance information. The cells can include living, dead, or fixed cells or fractions of cells. The present invention enables, *inter alia*, researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database.

According to the present invention, a computer program for identification and verification of biological properties of substances can include code that causes a sample of a substance to be administered to a cell. The code determines one or more features for two or more cell components, or markers, in the presence of the substance. The code can form one or more descriptors from the features. Descriptors can be formed by combining features of two or more cell components as identified using the markers. The code can then search one or more descriptors obtained from prior administered substances upon cells in order to locate descriptors having a relationship to the descriptors noted for the substance under study. The code predicts properties of the administered substance based upon the properties of the prior administered substances using the relationship between the descriptors. The code can provide for identifying properties of substances based upon effects on cell characteristics. Candidate drug mechanisms of action, potency, specificity, pharmacodynamic, and pharmacokinetic parameters, toxicity, and the like can be used as substance properties.

In a specific embodiment, the present invention provides a system for acquiring knowledge from cellular information. The system has a database comprising a database management module ("DBMS"). The system also has a variety of other modules, including a population module that is coupled to the DBMS and serves to categorize and store a plurality of features (including but not limited to cell size, distance between cells, cell population, as well as sub-cellular features such as organelle location, protein location and sub-cellular constituent location and

movement) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of a descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.

In a specific embodiment, the present invention provides a system for populating a database with cellular information. The system includes a cell holder (e.g., multi-well plate, chip, microfluidic assembly, or other cell chamber) comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. Note – the light guide is one embodiment, but we don't want to be limited to it.

According to one embodiment, the present system also has an illumination apparatus including a liquid light guide operably coupled to the imaging device for highlighting the plurality of cells in a relatively even spatial manner for image capturing and measurement purposes. Still further, the liquid light guide allows sub-elements (e.g., filter, lamp) of the illumination apparatus to be placed at a remote location to prevent mechanical interference of the cell holder during image capturing. Alternative lighting methodologies may, with equal facility, be implemented.

The system also has an image-capturing device (e.g., charge coupled device camera, translation stage, shutter, microscope, software, shutter control) coupled to a computing device (e.g., computer, network computer, work station, analog computing device, on-board image-processor, and laptop). The image-capturing device is adapted to capture at least one image in at least one of the plurality of sites. One some embodiments, multiple images can be captured, where each image represents a different cell component (or portion). The image-capturing device can be adapted to convert the image into a digital representation, which highlights the feature or features of the one site.

A database storage device (e.g., relational database, object oriented database, mixed object oriented database) includes a database management element. The

database is coupled to the image capturing device. In a specific embodiment, the present system includes modules for feature extraction, generation of descriptions, and data preparation and analysis.

In a specific embodiment, the present invention provides a novel system for determining an effect of a manipulation of a cell using one or more image frames. The system has a plate comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. The system also has an image capturing device to capture a plurality of images of at least one site from the plurality of sites. The image capturing device is coupled to the computing device. The system also has an image processing device to combine the plurality of images of at least one site or plurality of sites. The image processing device is operably coupled to the plate. An image processing device is also included. The image processing device can be adapted to form a digitized representation of the plurality of images from the site or plurality of sites. Furthermore, the system has a database storage device comprising a database management element. The database can be adapted to retrieve the descriptor or descriptors of the plurality of features from the computing processing device and storing them in a selected manner.

In a specific embodiment, the present invention provides a system for capturing cellular information. The system also has an image acquisition system comprising a charged coupled device camera adapted to capture an image of a plurality of manipulated cells in various stages of the cell cycle. The stages of the cell cycle are currently understood to include interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase. The principles of the present invention specifically contemplate the application thereof on additional cell cycle stages when and if they are identified.

An optical source is coupled to the image acquisition system for highlighting the plurality of manipulated cells in the various stages of the cell cycle. The illumination apparatus provides for an acquisition of the image of the plurality of manipulated cells. In a specific embodiment, the illumination apparatus has a liquid light guide coupled to a light source at a remote location.

A variety of user interfaces are utile for accessing the several features of the present invention. Those having ordinary skill in the art will appreciate that different user interfaces may be required to support different research scenarios. The

present invention specifically contemplates the utilization of a wide variety of user interfaces.

Numerous benefits are achieved by way of the present invention over conventional techniques. The present invention can provide techniques for predictive cellular bioinformatics that can streamline a number of important decisions made in the drug discovery industry. The present invention can be implemented using off the shelf hardware including databases. In other aspects, the present invention can find useful information about substances as well as cells or portions of cells. Furthermore, the present invention can acquire more then one feature using more than one manipulation. Moreover, the present invention can provide information about a wide variety of cellular information that is not conventionally available. This information includes information about different cell components, e.g., nuclei and Golgi apparatus. Still further, the present invention provides an automated or semi-automated technique for acquiring images and populating a database. The present database can be combined with others such as genomics, and the like. Moreover, the present invention can be implemented to predict, *inter alia*, a mechanism of action, toxicity, target validation, and pre-clinical disease model.

A further understanding of the nature and advantages of the invention herein may be realized by reference to the remaining sections of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWING

For more complete understanding of the present invention, reference is made to the accompanying Drawing in the following Detailed Description of the Invention. In the drawing:

Fig. 1 is a simplified system diagram according to an embodiment according to the present invention;

Figs. 1A-1B are more detailed diagrams of database systems according to embodiments of the present invention;

Fig. 2 is a simplified block diagram according to an alternative embodiment according to the present invention;

Figs. 3-6 are simplified diagrams of system elements according to embodiments of the present invention;

Figs. 7A-7K illustrate representative block diagrams of simplified process steps in a particular embodiment according to the present invention;

Fig. 8A-8F illustrate representative quantified descriptors of effects of manipulations on images of cells in a particular experiment;

Fig. 9 illustrates example images for different types of morphologies in a particular experiment;

Fig. 10 illustrates a distribution of various morphologies in a cell population responsive to drug concentration in a particular experiment;

Fig. 11 illustrates a graph of quantified features of effects of manipulations on cells in a particular experiment;

Fig. 12 illustrates effects of external agents on cells in a particular experiment;

Fig. 13 illustrates 4 panels for each marker for a plurality of A549 cells in a particular experiment;

Fig. 14 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells in a particular experiment;

Fig. 15 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 20x in a particular experiment;

Fig. 16 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 40x in a particular experiment;

Fig. 17 illustrates a representative input for a morphometric analysis program in a particular embodiment according to the present invention; and

Figs. 18-19 illustrate examples of the generation of pseudo-sequences and clustering in a particular embodiment according to the present invention.

Fig. 20 is a block diagram for a first research scenario;

Fig. 21 is a block diagram for a second research scenario; and

Fig. 22 is a block diagram for a third research scenario.

Reference numbers refer to the same or equivalent parts of the invention throughout the several figures of the Drawing.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, techniques for determining information about manipulated cells or substances based upon living, fixed, or dead cell structures or portions of cells are provided. In an exemplary embodiment, the present invention provides a novel system including computer codes coupled to a database and user interfaces for acquiring, storing and retrieving such information. Other embodiments provide a novel image capturing system for providing digitized representations of live and dead cell structures or the like.

Fig. 1 is a simplified system diagram 10 of a cellular knowledge-based system according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present system 10 includes a variety of elements such as a computing device 13, which is coupled to an image processor 15 and is coupled to a database 21. The image processor receives information from an image capturing device 17, which image processor and image capturing device are collectively referred to as the imaging system herein. The image capturing device obtains information from a plate 19, which includes a plurality of sites for cells. These cells can be biological cells that are living, fixed, dead, cell fractions, cells in a tissue, and the like. The computing device retrieves the information, which has been digitized, from the image processing device and stores such information into the database. A user interface device 11, which can be a personal computer, a work station, a network computer, a personal digital assistant, or the like, is coupled to the computing device.

Fig. 1A is a simplified diagram of a database system 1000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. Database system 1000 includes a variety of techniques for processing images from biological cells, e.g., fixed, living, and dead cells, and cell portions. As shown, images are acquired 1001. These images can be from a single frame or multiple frames. As merely an example, an image processing system may analyze such images. One example of

such an image processing system is described below, but should not be construed as limiting certain claims.

In a specific embodiment, cell samples are manipulated using a compound (e.g., substance, drug). The cell samples are imaged for a simple portion or portions, e.g., manipulated cell substructure, manipulated spatial feature of cell, cell density. Image processing techniques are used to extract 1003 the feature or features from the image or images. The features can be an independent or a dependent set of cell characteristics (which may be predominately visual) including, for example, count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface, average intensity, total intensity, optical density, radial dispersion, texture difference, and others. Each of these features corresponds to a similar manipulation by a compound. Each manipulation forms a new set of features, which are identifiable to the compound. Once each set of features has been extracted, the feature set is populated 1004 into a database 1012. Accordingly, the database includes many sets of features, where each set corresponds to a different manipulation for a selected cell. Each set of features corresponding to a manipulation provides a descriptor 1009, which is also stored 1019 in the database. The descriptor is a "finger print" including each feature for the manipulation. Each descriptor may be unique, or may have similarities to other descriptors or may even be the same as other descriptors for known and unknown manipulations.

The present system retrieves features, which we define as simple features herein, and forms composite features 1007 from them. More than one feature can be combined in a variety of different ways to form these composite features. In particular, the composite feature can be any function or combination of a simple feature and other composite features. The function can be algebraic, logical, sinusoidal, logarithmic, linear, hyperbolic, statistical, and the like. Alternatively, more than one simple feature can be combined in a functional manner (e.g., arithmetic, algebraic). As merely an example, the composite feature equals a sum of feature 1 and feature 2, where these features correspond to the same manipulation. Alternatively, the composite feature equals feature 1 divided by feature 2. Alternatively, the composite feature equals feature 1 minus feature 2. Alternatively,

the composite feature equals a constant times feature 1 plus feature 2. Of course, there are many ways that the composite feature can be defined. The present system also stores 1017 these features in the database. The composite features can also be further combined with simple features. Once these features are defined as descriptors, they are stored 1019 in the database.

Fig. 1B is a simplified diagram of a database system engine 2000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. The engine can be implemented into the present database for populating, searching, and predicting compound or cell characteristics. As merely an example, engine 2001 includes an input/output module 2008. The input/output module is used to input and output information from the database. The information includes, among others, a plurality of feature sets, which correspond to many manipulations. Additionally, the information includes descriptors, which each corresponds to a set of features from the manipulation. The database also has a population module, which is used to configure the features based upon an entity relationship, which has been predetermined.

The database engine also has other modules. In particular, the database has a transcription module, which transfers a preselected set of features and creates a descriptor from them. The transcription module can be used to take a known compound, which has features, to transcribe them into a descriptor. Alternatively, the transcription module can be used to take an unknown compound, which has features, to transcribe them into a descriptor. These descriptors are provided into the database for subsequent use. Finally, the database engine has a prediction module, which can be used to potentially predict a property (e.g., mechanism of action) of an unknown compound. Here, the unknown compound is provided with a descriptor, but the property of the compound is unknown. In one embodiment, the prediction module compares a descriptor of an unknown compound with the many descriptors of known compounds, which were in the populated database. Depending upon the matching criteria, the prediction module will attempt to uncover one or more descriptors of known compounds. Once the prediction module finds the descriptors of the known compounds based upon the descriptor for the unknown compound, it identifies a potential property of such unknown compound for analysis and review. Here, it is

believed that certain features of the known compound, which are similar to those features of the unknown compound may uncover a property to the unknown compound. Details of the present software engine are described more fully below.

Fig. 2 is a simplified block diagram 20 of a cellular knowledge-based system according to an alternative embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Like reference numerals are used in the present diagram as the previous diagram for easy cross-referencing, but are not intended to be limiting in any manner. The present diagram 20 includes a variety of elements such as a processor 13 or computing device coupled to a database 11. The processor can be used for retrieving and storing information from the database. The system also includes a plurality of system elements. such as a cleaner 23, a dispenser 25, and an image capturing system 27, which are also coupled to the database in some embodiments. These elements can be coupled to each other through a network or the like. As merely an example, the network can be a NetWare™ network from Novell Corporation or an internet network or the Internet but can also be others and any combination thereof. The system also has an output device 31, which can be used to output information from the database, processor, or other system elements. Details of these elements are described more fully below in reference to the Figs.

Figs. 3-5 are simplified drawings of system elements according to embodiments of the present invention. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. As merely an example, Fig. 3 is a simplified diagram of a processor or computing device 13. The computing device 13 includes a bus 112 which interconnects major subsystems such as a central processor 114, a system memory 116 (e.g., random access memory), an input/output ("I/O") controller 118, an external device such as a display screen 124 via a display adapter 126, a keyboard 132 and a mouse 146 via an I/O controller 118, a SCSI host adapter (not shown), and a floppy disk drive 136 operative to receive a floppy disk 138.

The computing device has other features. Storage Interface 134 may act as a storage interface to a fixed disk drive 144 or a CD-ROM player 140 operative

to receive a CD-ROM 142. Fixed disk 144 may be a part of computing device or may be separate and accessed through other interface systems. A network interface 148 may provide a direct connection to a remote server via a telephone link or to the Internet. Network interface 148 may also connect to a local area network ("LAN") or other network interconnecting many computer systems. Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 3 to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 3. The operation of a computer system such as that shown in Fig. 3 is readily known in the art and is not discussed in detail in this application. Computer code to implement the present invention, may be operably disposed or stored in computer-readable storage media such as system memory 116, fixed disk 144, CD-ROM 140, or floppy disk 138. The computer code can be organized in terms of processes or modules, depending upon the application. That is, the computer code can include a prediction module, a translation, module, or other modules to carryout the functionality described herein, as well as others.

Figs. 4 and 5 are simplified diagrams of an imaging system 200 according to an embodiment of the present invention. As shown, the imaging system 200 includes a variety of features such as housing 203, which holds a stage assembly 204. The stage assembly includes an x-stage movement element 206, which is along an x-direction, and a y-stage movement element 207, which is along a y-direction. The imaging system also includes a z-direction movement element, which is perpendicular to the x-y plane. The z-direction movement motor can be attached to the stage, or to the objective nosepiece by way of the microscope housing, or as an external motor between the objective and the microscope housing. The stage can align in any one of the directions to an accuracy of one micron and less, or one-half micron and less, or one-quarter micron and less, depending upon the embodiment.

The stage holds a plate 202 or cell holder, which houses one of a plurality of samples. The plate includes a spatial array 209 of process sites. Each of the process sites can include a plurality of cells and solutions depending upon the embodiment. Each of the sites can carry a sufficient amount of solution to prevent substantial evaporation of the sample during processing in some embodiments. In embodiments for large scale analysis, the plate includes at least 96 sites, or more than

or equal to 384 sites, or more than or equal to 1,536 sites. The plate bottom is transparent and thin, which allows light to pass through the sample. Additionally, the plate is made of a suitable chemical resistant material. As merely an example, the plate can be either a 96, or 384, or 1536 or other formats from places such as Becton Dickinson of Franklin Lakes, NJ, or Corning Science Products of Corning, NY. In a preferred embodiment, the plate is a Corning Costar black-walled 96 well plate catalog #3904 from Corning Science Products of Corning, NY, but should not be limited to these in some applications, but can be others.

Also shown is the condenser for the microscope 201, which can be used to collect phase, DIC, or bright field images of the cells. Images resulting from the illumination of the samples to fluorescence, phase, DIC, or bright field techniques are collected using an image capturing device 208, which captures an image or images of cells from the plate. In a specific embodiment, the microscope is an inverted configuration with the objectives on the bottom of the plate and the condenser disposed overlying an upper surface of the sites, while the image capturing device underlies the sites. Images captured by the imaging device, whether analogue or digital, are viewed by a monitor or other devices. The image capturing device can be any camera assembly such as a charge coupled device camera, which is known as a CCD camera, or other high resolution camera capable of capturing images from the sites. In a specific embodiment, the camera is an interline CCD camera which does not require an external shutter.

In a specific embodiment, the present imaging system can be any suitable unit that is flexible for automated image collection using multi-well plastic plates. The imaging system also should be adapted to collect high-resolution images of cells on plastic or glass plates, cell growth chambers, or coverslips. The system also can be used for imaging multiple cell markers in multiple imaging conditions. To accomplish this, the microscope system has a variety of elements such as a light source, a motorized excitation filter wheel and shutter, x-y-z-motorized stage, excitation and emission filters, Fluor phase and DIC objectives, motorized objective nosepiece, dichroic filters, motorized dichroic filter cubes, phase and DIC rings and prisms, CCD camera, and software control. As merely an example, the present imaging system can have components such as those listed in the Table below.

DESCRIPTION	MAKER	MODEL
Microscope	Zeiss	100M
(x-y) motorized stage	Prior	
Xenon lamp	Sutter	Lambda
Filter wheel	Sutter	Lambda-10
Microtitre Plate holder	Prior	500-H223R
Isolation Table	Kinetic Systems	9101-24-85
Objective Spacers	Polytec PI	P-721.90
Camera	Hamamatsu	C47-95
Computer	IBM	IntelliStation
Software	Metamorph	v.4
Objectives	Zeiss	Achroplan 10x/0.25 LD-Achroplan 20x/0.4 LD-Achroplan 40x/0.6

Table: Image Acquisition System Elements

In a specific embodiment, the present system has the following capabilities, which are not intended to be limiting.

Image acquisition

1) Ability to automatically acquire multi-wavelength images from multiple sites on one multi-well plate, to sequentially name image files, and to log any imaging parameter information with image files.

2) Ability to link images with a larger database/spreadsheet of information.

3) Ability to automatically collect multiple plates by interfacing the imaging system with a robotic arm.

X-Y control

1) Ability to place 96, 384, or 1536 well plates onto microscope stage and move to each well sequentially.

2) Ability to return to each well and collect another round of images (multi-site time-lapse) or ability to collect rapid time-lapse information at each well (time-lapse of many wells).

3) Ability to collect a low magnification image, automatically determine features which may be of interest, automatically change the objective to a higher magnification, and collect high magnification images of a fixed number of those identified cells in the sample.

4) Ability to collect multiple frames in each site.

Z control

1. Ability to auto-focus with substantially minimal damage to biological specimen or fluorophore.

2. Ability to auto-focus rapidly.

The present embodiment of the imaging system is shown by way of Figs. 5A and 5B. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present imaging system 40 includes a variety of elements such as a microscope 41, which is preferably an epi-fluorescent microscope, but can be confocal, multiphoton, or hybrid types. The microscope includes elements 41A, the motorized Z-axis; 41B, the motorized dichroic filter cube holder; and 41C, the motorized objective nosepiece. In one embodiment, the microscope is a Model 100M made by Zeiss. The microscope communicates to computer 51 through control lines 73, 75, and 76. The imaging system also has camera 50 coupled to controller 50A and computing device 51, which oversees and controls operations of the elements of the imaging system.

The present microscope includes drivers for spatially moving a stage in two dimensions, including an x-direction, a y-direction, and moving the objective nosepiece in a z-direction in a Cartesian coordinate system. The z-direction movement is provided using a fast z-motor, which can make z-direction adjustments within a predetermined time. The z-direction movement generally provides for focussing of the sample to the camera. The focussing occurs within the predetermined time of preferably ten seconds and less, or five seconds and less, or one

second and less, depending upon the embodiment. As merely an example, the z-motor or positioner can be a model PIFOC objective nanopositioner made by a company called Physik Instrumente of Waldbronn, Germany, but also can be others. The z-motor couples to computer 51 through line 63, which may also include a controller. Depending upon the embodiment, a second z-motor 41A connected to the computer 51 by line 73 may be used to keep the z-motor 42 in the center of its travel. Alternatively, in other embodiments the stage could be provided with a z-motor allowing for movement of the stage in the z-direction.

The present stage also includes an x-y stage 43. The x-y stage moves plate 59, e.g., 96 site, 384 site, 1536 site. The x-y stage moves plate in an x-y spatial manner. The stage has an accuracy or repeatability of about 1 micron and less, or about 2 microns and less. The stage can move in a continuous manner or a stepped manner. The stage also can move up to 30 mm/sec. or faster. The stage also can move 1 mm/sec. and less, depending upon the embodiment. The stage can also step 0.1 micron and less or 1 micron and less, as well as other spatial dimensions. The stage can be one such as a Proscan Series made by Prior Scientific of Rockland, MA but can also be others. The stage is controlled via control line 61 through controller 43A, which couples to computer 51 through control line 65.

The stage includes plate holder 44. The plate holder can hold a single plate. In other embodiments, plate holder can also hold multiple plates. The plate holder can use mechanical, electrical, fluid, vacuum and other means for holding the plate or plates. The plate holder also is sufficiently stable for securing the plate. As merely an example, the plate holder is a Model 500-H223R made by Prior Scientific of Rockland, MA . In some embodiments, the plate holder may need adjustment in the z-direction to provide for a desirable focus of a sample on a plate. In these embodiments, the plate holder is supported by spacers 45 or a plurality of stage pins, which mechanically elevate the plate holder in the z-direction. These pins are generally made of a suitable material for supporting such plate holder and also are sufficiently resistant to chemicals and the like.

In some embodiments, the entire imaging system is placed on an isolation table 49. The isolation table is disposed between the microscope and support structure. The isolation table is designed to prevent excessive vibration of the plate. The isolation table is made of a suitable material such as steel and honeycomb but can

be others. The table has a thickness of about 8 inches or preferably less than about 24 inches. In one embodiment, the table is Model 9101-24-85 made by Kinetic Systems of Boston, MA.

The imaging system also has a lamp or illumination assembly 62. The lamp assembly provides for a light source (See reference letter B) to a plurality of elements in the imaging system. For easy reading, the light path is defined by the doted lines, which are not intended to be limiting. The lamp assembly has a variety of elements such as a Xenon lamp 46. The Xenon lamp provides light at about 320 to 700 nanometers (Prefocused). The Xenon lamp is 175 or 300 Watts. As merely an example, the lamp can be a Lambda Model made by Sutter Instrument Company of Novato, CA.

Referring to Fig. 5B, the lamp assembly also has a cold mirror 58, an excitation filter wheel 48, excitation filter(s) 55, and an excitation light shutter 57. As shown, light is derived from the Xenon lamp, reflects off of the cold mirror 58, traverses through the excitation filter or filters 55, and is controlled by the excitation light shutter 57. The lamp assembly has filter wheel 48, which houses one of a plurality of filters, including excitation filters. The shutter and filter wheel are controlled via control lines 67, which are coupled to a computer 51 or other type of computing device. The control lines 67 are coupled through controller 57A (for element 57) and controller 48A (for element 48) via control line 69 to computer 51.

Preferably, light traverses from the lamp assembly through a light guide 47 to illuminate features within the plate. The light guide is suitably selected to have a flexible member, which can be used to place lamp source at a remote location away from the imaging device. The flexible member substantially keeps any vibration from the lamp assembly away from the imaging device. In some embodiments, the member is at least 1 foot away from the imaging device. The light guide is a guide, which is a flexible hose-type sleeve. The sleeve is filled with a liquid such as an aqueous solution containing chloride or phosphate. A thin layer may be formed on the inside of the sleeve. The layer can be a containing tetrafluoroethylene and mexafluoropropylene, or containing tetrafluoroethylene and perfluoromethyl vinyl ether, or tetrafluoroethylene and perfluoropropyl vinyl ether. An example of such a light guide is described in International Application No. WO/98/38537 filed February 29, 1997, and assigned to NATH, Gunther. The liquid

light guide has less than about 30% transmission loss of the light at a remote location such as the imaging system.

Light is derived from the lamp assembly and directs off of filter 56, which directs the light upward. Filter 56 can be a dichroic and emission filter, as well as others. The light traverses through microscope nosepiece 41C, and traverses through objective spacers 54. An objective 53 magnifies the light toward a predetermined point on the plate 59. The objective can be, for example, made by Zeiss of Jena, Germany, as well as other companies. The objective can be one of a plurality including 1X, 10X, 20X, 40X, and others, depending upon the application. Magnification can be further expanded or contracted by intermediate optics between the objective and the camera. Selection of filter or filters is controlled by computer 51 via control line 75.

The camera 50 captures an image of cells from plate 59. The image is obtained from light scattering off of cells or portions of cells in the plate through objective 53, through objective spacers, through filters 56, which are captured at camera 50. In this preferred embodiment, the camera is a digital camera, but can be an analogue camera. The digital camera is a CCD camera, which has 1280 by 1024 pixels, or more or less. The pixels can be 6.7 microns in dimension or more or less. The camera preferably is substantially free from an external shutter to quickly capture a plurality of images of cells from the plate. The camera is controlled via control line 71 through controller 50A, which connects to computer 51 through control line 70. The present invention can also include other types of image acquisition devices selected from at least an epifluorescence, a confocal, a total-internal reflection, a phase, a Hoffman, a bright field, a dark field, a differential interference contrast, an interference reflection, or multi-photon illumination device.

The present imaging system stores images on a high density memory device 60. The high density memory device is preferably optical, but can also be magnetic. The high density memory device can be any suitable unit that is capable of storing a plurality of images from a plurality of sites in the plate. The memory device can be a compact disk, which would generally use a compact disk burner or the like. Depending upon the embodiment, the high density memory device is used to archive the images that are captured from the camera in the imaging system. Further details

of the imaging system can be found throughout the present specification, and more particularly below.

As merely an example, the present invention can be implemented using the following sequence of steps, which have been described in a journal entry form. Here, images are opened and objects are identified based on a background value that has been edited in starting image acquisition. Information is maintained in a spreadsheet or other database format, which has the following information for each object:

Image Name	Image Plane	Image Date and Time
Elapsed Time	Object #	Total area
Pixel area	Area	Hole area
Relative hole area	Standard area count	Perimeter
Length	Breadth	Fiber length
Fiber breadth	Shape factor	Ell. form factor
Inner radius	Outer radius	Mean radius
Average gray value	Total gray value	Optical density
Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis
EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major Axis Angle	EFA Harmonic 2, Ellipse Area
EFA Harmonic 2, Axial Ratio	EFA Harmonic 3, Semi-Minor Axis	

After computations are done, the log file is saved. In particular, the file is saved in an appropriate place with an appropriate name.

In a specific embodiment, the present invention provides the following detailed example of journal entries, which should not limit the scope of the invention.

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)
Stage (Log Position)	
Stage (Scan Wells)	User picks wells to scan: runs 3x3 image collection.jnl.

3X3 IMAGE COLLECTION.jnl

Stage (Scan)	Takes 9 images of well, -1600 motor steps apart from left to right 3 columns and 3 rows, runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL.

FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl.

Stage (Log Position)	Logs stage position of each image
ADC – Focus	Opens up the manual focusing window with whatever focus time is current set
Show Message and Wait	Interactive: user hits enter to continue when done focusing

ADC-Acquire from Digital Camera	Takes Hoechst image
Save Using Sequential File Names	
Close	Closes image window

START IMAGE ANALYSIS.jnl

Low Pass	3x3 convolution of already opened image
Low Pass	3x3
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 4. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 3. into the constant Value field
Threshold image	Creates threshold 1 unit above 0 to 4096
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 8.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 7. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS.jnl step 3
Threshold Image	1 unit above 0
Integrated Morphometry – Load State	Hoecsht.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

Log obj and sum data.jnl

Integrated Morphometry – Log Data	Logs object data into Sheet 1
Integrated Morphometry – Log Data	Log summary data into Sheet 2

COLLECT AUTOMATED IMA DATA INONE SPREADSHEET.jnl

Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Loops IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save Excel spreadsheet

OPEN OBJECT LOG DDE FILE.jnl

Open Object Log	Opens a DDE object log into sheet 1 of an Excel spreadsheet
Open Summary Log	Opens a summary log into sheet 2

COLLECT AUTOMATED IMA DATA INONE SPREADSHEET 16 BIT IMAGES.jnl

Arithmetic	Interactive: Opens Arithmetic window for user to input background subtraction level from START IMAGE ANALYSIS.jnl step 3
Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Interactive: Runs IMA OBJECTS 16 bit.jnl. User picks directory from which to choose.

IMA OBJECTS 16bit.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Copy to 8-bit Image	No autoscale, to new untitled image
Save Using Sequential File Name	Saves 8bit image using previously defined Sequential File names.
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS16 TO 8 BIT.jnl step 5
Threshold Image	1 unit above 0 to 255

Integrated Morphometry – Load State	Hoecsht.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

START IMAGE ANALYSIS 16 to 8 BIT.jnl

Copy to 8-bit Image	No autoscale, to new untitled image
Close	Closes 16 bit image
Low Pass	3x3 convolution
Low Pass	3x3 convolution
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 6. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 5. into the constant Value field
Threshold image	Creates threshold by 1 unit above 0 to 255
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 10.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 9. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS WITH NEW LOG FILE.jnl

Run Journal	OPEN OBJECT LOG DDE FILE.JNL
Run Journal	IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save every Excel spreadsheet generated.

INTERACTIVE IMA OBJECTS.jnl

Threshold Image	User manually sets threshold
Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Objects
Integrated Morphometry – Log Data	Into open object.log file

COLLECT INTERACTIVE IMA DATA.jnl

Close Object Log	
Open Object Log	Interactive
Annotate Log File	Interactive: experimental information that will go into the first line of the object log file
Loop for all Images in Directory	Runs INTERACTIVE IMA OBJECTS.jnl

CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

Stage (Log Position)	
ADC-Focus	

Show Message and Wait	Interactive – user presses Enter when done focusing
ADC – Acquire from Digital Camera	Hoechst
Save Using Sequential File Name	
Close	Close open image

COLLECT HOECHST AND FITC.jnl

Run Journal	FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL
Run Journal	CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

3X3 IMAGE COLLECTION HOECHST FITC.jnl

Stage (Scan)	COLLECT HOECHST AND FITC.jnl

AUTOMATED 3X3 IMAGE COLLECTION HOECHST FITC.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Excel DDL files
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)

Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs 3X3 IMAGE COLLECTION HOECHST FITC.jnl

AUTOMATED IMAGE COLLECTION.jnl

Sct Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL. Well to well travel = (-9035, -9035)

STARTUP.jnl

Install and Configure Devices	Open Stage Meta Devices
Set Live Video Channel	

Preferences	Measure Objects: Draw failed classifier objects, Exclude objects that touch the edge of the image, Enable Elliptical Fourier Parameters, **turn off** Warn users when measurement data will be erased Image Saving: Save Tiff/stk using LZW compression Image Windows: Use transparent thresholds.
Configure Default Paths	C:\Metamorph Data C:\Metamorph Data\Commmon Settings
Load Journal Taskbar	Common.JTB

Nested Journals

Automated 3x3 Image Collection

Loop 3x3 image collection

Loop focus, collect image, save sequential file name

Automated 3x3 image collection Hoechst FITC

Loop 3x3 image collection Hoechst FITC

loop Collect Hoechst and FITC

focus, collect image, save sequential file name

change filter, collect image, save sequential file name

Automated image collection

Loop focus, collect image, save sequential file name

Collect automated IMA data in one Spreadsheet

Open object log DDE file

Loop IMA objects

Log obj and sum data

Collect automated IMA data in one spreadsheet 16 bit images

Open object log DDE file

Loop IMA objects 16 bit

Log obj and sum data

Although the above has been generally described in terms of a specific user interface and software code, other user interfaces and code can also be used. One of ordinary skill in the art would recognize many other variations, alternatives, and modifications.

Fig. 6 is a simplified diagram 600 of a cleaning and dispensing system according to an embodiment of the present invention. This system 600 includes a variety of elements such as a dispensing head 609, which is coupled to a plurality of pipettes 601. The pipettes input and output fluids or solutions from plate 603. The plate has a plurality of sites, each of which can be used to input cells or a combination of cells and solution. The system also has elements to house solutions 605, which are used to manipulate cell samples in the plate. The dispensing head is supported through a support member 607, which is sufficiently rigid to allow for movement of the head. The dispenser is coupled to the present system in a mechanical and electrical manner, which provides for a fully integrated system for providing cell samples to the imaging system according to the present invention.

Fig. 7A illustrates a representative block flow diagram of simplified process steps of a method for determining properties of a manipulation based upon effects of the manipulation on one or more portions of one or more cells in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. In step 700, one or more samples of cells can be provided. These cells can be live, dead, or fixed cells, or cell fractions. The cells also can be in one of many cell cycle stages, including G0, G1, S, G2 or M phase, M phase including the following cell cycle stages: interphase, prophase, prometaphase, metaphase, anaphase, and telophase.

Cell components tracked in presently preferable embodiments can include proteins, protein modifications, genetically manipulated proteins, exogenous proteins, enzymatic activities, nucleic acids, lipids, carbohydrates, organic and inorganic ion concentrations, sub-cellular structures, organelles, plasma membrane, adhesion complex, ion channels, ion pumps, integral membrane proteins, cell surface receptors, G-protein coupled receptors, tyrosine kinase receptors, nuclear membrane receptors, ECM binding complexes, endocytotic machinery, exocytotic machinery, lysosomes, peroxisomes, vacuoles, mitochondria, Golgi apparatus, cytoskeletal filament network, endoplasmic reticulum, nuclear membrane, proteosome apparatus, chromatin, nucleolus, cytoplasm, cytoplasmic signaling apparatus, microbe specializations and plant specializations.

The following table illustrates some markers and cell components commonly used by embodiments according to the present invention. Other markers can be used in various embodiments without departing from the scope of the invention.

Cell component	Marker	Disease State
Plasma membrane (including overall cell shape)	Carbocyanine dyes Phosphatidylserine Various lipids Glycoproteins	Apoptosis-Cancer Apoptosis-Neural degenerative Ds
Adhesion complexes	Cadherins Integrins Occludin Gap junction ERM proteins CAMs Catenins Desmosomes	Thrombosis Metastasis Wound healing Inflammatory Ds Dermatologic Ds
Ion Channels and Pumps	Na/K Atpase Calcium channels Serotonin reuptake pump CFTR	Cystic fibrosis Depression Congestive Heart Failure Epilepsy

G coupled receptors	β adrenergic receptor Angiotensin receptor	Hypertension Heart Failure Angina
Tyrosine kinase receptors	PDGF receptor FGF receptor IGF receptor	Cancer Wound healing Angiogenesis Cerebrovascular Ds
ECM binding complexes	Dystroglycan Syndecan	Muscular Dystrophy
Endocytotic machinery	Clathrin Adaptor proteins COPs Presenilins Dynamin	Alzheimer's Ds
Exocytotic machinery	SNAREs Vesicles	Epilepsy Tetanus Systemic Inflammation Allergic Reactions
Lysosomes	Acid phosphatase Transferrin	Viral diseases
Peroxisomes/Vacuoles		Neural degenerative Ds
Mitochondria	Caspases Apoptosis inducing factor F1 ATPase Fluorescein Cyclo-oxygenase	Apoptosis Neural degenerative Ds Mitochondrial Cytopathies Inflammatory Ds
Golgi Apparatus	Lens Culinaris DiOC6 carbocyanine dye COPs	

Cytoskeletal Filament Networks	Microtubules Actin Intermediate Filaments Kinesin, dynein, myosin Microtubule associated proteins Actin binding proteins Rac/Rho Keratins	Cancer Neural degenerative Ds Inflammatory Ds Cardiovascular Ds Skin Ds
Endoplasmic Reticulum	SNARE PDI Ribosomes	Neural degenerative Ds
Nuclear Membrane	Lamins Nuclear Pore Complex	Cancer
Proteosome Apparatus	Ubiquityl transferases	Cancer
Chromatin	DNA Histone proteins Histone deacetylases Telomerases	Cancer Aging
Nucleolus	Phase markers	
Cytoplasm	Intermediary Metabolic Enzymes BRCA1	Cancer
Cytoplasmic Signaling Apparatus	Calcium Camp PKC pH	Cardiovascular Ds Migraine Apoptosis Cancer
Microbe Specializations	Flagella Cilia Cell Wall components: Chitin synthase	Infectious Ds

Plant specializations	Choloroplast Cell Wall components	Crop Protection

Then, in a step 702, one or more samples of the manipulation can be provided to the cells. Manipulations can comprise one or any combination of chemical, biological, mechanical, thermal, electromagnetic, gravitational, nuclear, or temporal factors, for example. For example, manipulations could include exposure to chemical compounds, including compounds of known biological activity such as therapeutics or drugs, or also compounds of unknown biological activity. Or exposure to biologics that may or may not be used as drugs such as hormones, growth factors, antibodies, or extracellular matrix components. Or exposure to biologics such as infective materials such as viruses that may be naturally occurring viruses or viruses engineered to express exogenous genes at various levels. Bioengineered viruses are one example of manipulations via gene transfer. Other means of gene transfer are well known in the art and include but are not limited to electroporation, calcium phosphate precipitation, and lipid-based transfection. Manipulations could also include delivery of antisense polynucleotides by similar means as gene transfection. Other genetic manipulations include gene knock-outs or gene mutations. Manipulations also could include cell fusion. Physical manipulations could include exposing cells to shear stress under different rates of fluid flow, exposure of cells to different temperatures, exposure of cells to vacuum or positive pressure, or exposure of cells to sonication. Manipulations could also include applying centrifugal force. Manipulations could also include changes in gravitational force, including sub-gravitation (the preferred embodiment in outer space). Manipulations could include application of a constant or pulsed electrical current. Manipulations could also include irradiation. Manipulations could also include photobleaching which in some embodiments may include prior addition of a substance that would specifically mark areas to be photobleached by subsequent light exposure. In addition, these types of manipulations may be varied as to time of exposure, or cells could be subjected to multiple manipulations in various combinations and orders of addition. Of course, the type of manipulation used depends upon the application.

Then, in a step 704, one or more descriptors of a state in the portions of the cells in the presence of the manipulation can be determined using the images

collected on the imaging system. Descriptors can comprise scalar or vector values, representing quantities such as area, perimeter, dimensions, intensity, gray level, aspect ratios, and the like. Other types of descriptors include, but are not limited to, one or any combination of characteristics such as a cell count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average intensity, a total intensity, and an optical density. These descriptors can be average or standard deviation values, or frequency statistics from the descriptors collected across a population of cells. These descriptors can be further reduced using other methods such as principal component analysis and the like. In some embodiments, the descriptors include features from different cell portions or cell types. That is, a first feature can be from a nuclei and a second feature is from another cell structure such as Golgi apparatus, mitochondria, spacing between cell structures or cells themselves, as well as many others.

A presently preferable embodiment uses descriptors selected from the following table. Other descriptors can also be used without departing from the scope of the invention.

Name of Parameter	Explanation/Comments
Count	Number of objects
Area	
Perimeter	
Length	X axis
Width	Y axis
Shape Factor	Measure of roundness of an object
Height	Z axis
Radius	
Distribution of Brightness	
Radius of Dispersion	Measure of how dispersed the marker is from its centroid
Centroid location	x-y position of center of mass
Number of holes in closed objects	Derivatives of this measurement might include, for

	example, Euler number (= number of objects - number of holes)
Elliptical Fourier Analysis (EFA)	Multiple frequencies that describe the shape of a closed object
Wavelet Analysis	As in EFA, but using wavelet transform
Interobject Orientation	Polar Coordinate analysis of relative location
Distribution Interobject Distances	Including statistical characteristics
Spectral Output	Measures the wavelength spectrum of the reporter dye. Includes FRET
Optical density	Absorbance of light
Phase density	Phase shifting of light
Reflection interference	Measure of the distance of the cell membrane from the surface of the substrate
1,2 and 3 dimensional Fourier Analysis	Spatial frequency analysis of non closed objects
1,2 and 3 dimensional Wavelet Analysis	Spatial frequency analysis of non closed objects
Eccentricity	The eccentricity of the ellipse that has the same second moments as the region. A measure of object elongation.
Long axis/Short Axis Length	Another measure of object elongation.
Convex perimeter	Perimeter of the smallest convex polygon surrounding an object
Convex area	Area of the smallest convex polygon surrounding an object
Solidity	Ratio of polygon bounding box area to object area.
Extent	proportion of pixels in the bounding box that are also in the region
Granularity	
Pattern matching	Significance of similarity to reference pattern
Volume measurements	As above, but adding a z axis

Then, in a step 705, a database of cell information can be provided. Next, in a step 706, a plurality of descriptors can be searched from a database of cell information in order to locate descriptors based upon one of the descriptors of the manipulation. Then, in a step 708, properties of the manipulation are predicted based upon the properties of the located descriptors. Properties can comprise toxicity, specificity against a subset of tumors, mechanisms of chemical activity, mechanisms of biological activity, structure, adverse biological effects, biological pathways, clinical effects, cellular availability, pharmacological availability, pharmacodynamic properties, clinical uses and indications, pharmacological properties, such as absorption, excretion, distribution, metabolism and the like.

In a particular embodiment, step 706 comprises determining matching descriptors in the database corresponding to a prior administration of the manipulation to the descriptors of the present administration of the manipulation. In a particular embodiment according to the present invention, combinations of measurements of scalar values can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell-substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be analyzed, classified, and compared using a plurality of techniques, such as statistical classification and clustering, heuristic classification techniques, a technique of creating "phylogenetic trees" based on various distance measures between descriptors from various drugs. In this embodiment, numeric values for the descriptors can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound descriptor with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphological properties of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, comparisons can be performed on acquired image

features. Some embodiments can comprise statistical and neural network - based approaches to perform comparisons of various features. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data.

In some embodiments, classification, clustering and other types of predictive data analysis can be performed on features extracted from cell images. In a presently preferable embodiment, statistical procedures for comparisons, classification and clustering are performed on data obtained from imaging cells.

Fragments of data preparation and pre-formatting (S language):

```
>tmp.frame <- Generic.Summary
>names1 <- paste("Cell.line.5", tmp.names, sep=".")
> by.compound.matrix <- as.matrix(arranged.by.compound)
```

Example of the code for principal component analysis (data preparation) using S language:

```
all.data.princomp <- menuPrincomp(data =
by.compound.matrix, scores = T, cor = "Correlation",
na.action = T, print.short = T, print.importance = T,
print.loadings = T, cutoff.loadings = 0.1,
plot.screeplot = T, plot.loadings = T, plot.biplot = T,
plot.biplot.choices = c(1,2), predict.p = F)
```

Example of clustering using a divisive hierarchical clustering algorithm:

```
> div.hier.2.manhattan.cluster$call
diana(x = tmp.sum.by.comp, diss = F, metric =
"manhattan",
   stand = T, save.x = T, save.diss = T)
```

Another embodiment utilizes existing tools for biological sequence similarity searches, classification, and phylogenetic analysis . In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes according to a one of several sets of rules. Once

converted into a corresponding nucleotide or amino acid sequence representation, the fingerprints can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the PHYLIP (PHYlogeny Interference Package) a package of programs for inferring phylogenies (evolutionary trees) described in (Feldenstein, J. 1996 Methods Enzymol 266:418-427 and Feldenstein, J. 1981 J. Mol. Evol. 17(6):368-376).

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. Further details of a step of manipulation are noted more particular below.

Fig. 7B illustrates a representative block flow diagram of simplified process steps for determining one or more descriptors of a state in the portions of the cells in the presence of the manipulation of step 704 of Fig. 7A in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. In a step 712, an image of a cell portion is obtained. In some embodiments, the cell portion is visualized with a fluorescently labeled marker that is specific for the portion or portions of interest. A cell portion can include, for example, one or more of the following: nuclei, Golgi apparatus, and other features. The cell portion may vary in select embodiments according to the invention. Then, in a step 714, a digitized representation of the image obtained in step 712 is determined. In some embodiments, steps 714 and step 712 can comprise a single step. These embodiments use a digital imaging means such as a digital camera, to obtain a digital image of the target directly. Next, in a step 716, the digital representation of the image is processed to obtain image features. Image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Then, in a step 718 descriptors can be determined from the image features. Descriptors can comprise scalar or vector quantities and can comprise the image features themselves, as well as

composed features, such as shape factor derived by a relationship 4π * area / perimeter, and the like. Descriptors can also comprise statistical quantities relating to feature characteristics across a population of cells, such as a standard deviation, and average, and the like.

In a preferred embodiment, cells can be placed onto a microscope, such as a Zeiss microscope, or its equivalent as known in the art. A starting point, named Site A01, is identified to the microscope. A plurality of exposure parameters can be optimized for automated image collection and analysis. The microscope can automatically move to a new well, automatically focus, collect one or more images, at one or more wavelengths, move to a next well, and repeat this process for all designated wells in a multiple well plate and for multiple plates. A file having a size and an intensity distribution measurement for each color and rank for each well can then be created for the images acquired. Based on this information, a user or a computer can revisit sites of interest to collect more data, if desired, or to verify automated analysis. In a presently preferred embodiment, image automatic focus and acquisition can be done using computer software controlling the internal Z-motor of the microscope. Images are taken using a 10x, 20x, or 40x air long working distance objectives. Sometimes multiple images are collected per well. Image exposure times can be optimized for each fluorescent marker and cell line. The same exposure time can be used for each cell line and fluorescent marker to acquire data.

Fig. 7C illustrates a representative block flow diagram of simplified process steps for obtaining images of cell portions of step 712 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 720, a sample is provided to the imaging device. Samples can be provided in 96 well plates and the like. The sample may be loaded into a microscope, such as a Zeiss microscope or equivalent.

(2). In a step 722, a set of optical filters is selected to shine light of the appropriate wavelength to illuminate the first sample, which may be contained in a first well designated A01.

(3). In a step 724, an automatic focusing procedure is performed for the site. In a particular embodiment, the internal z-motor of the microscope which is attached to the objective nosepiece is used for automatic focusing of the microscope. In an alternative embodiments, the plate holding the samples is moved to perform automatic focusing of the microscope, or focusing can be performed by moving optical components attached to the microscope and the like.

(4). In a step 726, images are collected for the site. Images can be collected for every color at every site. Present embodiments can provide images for up to four colors. However, embodiments are contemplated that can provide more colors by using either a monochromator coupled with excitation filters which are on a filter wheel, or by digitally separating overlapping fluorophores. Those knowledgeable in the field will know that given calibration images of single fluorophores, a look-up table can be devised which will allow for the digital removal of fluorescence bleed-though of fluorescence which may occur in optical channels other than the one for which that filter has been optimized in instances of using more than one fluorophore at once. Cell growth and density information is also collected. Cell density is determined by what percentage of the area being imaged is inhabited by cells. In some embodiments, imaging can be facilitated using one or more biosensors, molecules such as non-proteins, i.e., lipids and the like, that are luminscently tagged. However, some embodiments can also use fluorescence polarization and the like. Fluorescence polarization is a homogeneous fluorescence technology where the excited state of the molecule lasts much longer than in normal fluorescence, taking seconds to minutes to reach equilibrium, obliterating the need to wash away fluorescence markers that are not specifically bound to a marker. Further, embodiments can detect differences in spectral shifts of luminescent markers. Some fluorescence markers, such as Nile Red sold by Molecular Probes of Eugene, OR, will change its emission peak wavelength depending on its environment. One can detect these changes by monitoring the level of fluorescence at both wavelengths and reading out at ratio of the two.

(5). In a step 728, a determination is made whether more fields of view need to be taken for a particular color. If this is so, then processing continues at step 726 at a new site. Otherwise, processing continues with a decisional step 730.

Images can now be taken by repeating step 726. In a preferred embodiment 4 to 9 images are collected at each site.

(5). In a step 730, a determination is made whether more optical configurations need to be taken in order to obtain images for all differently-marked cell portions the sample. If this is so, then in a step 732 a new optical configuration is determined. Images for the new optical configuration can now be taken by repeating steps 726 and 728.

(6). In a decisional step 734, after all optical configurations and images for fields of view in a sample have been obtained, a determination is made whether any further samples remain to be analyzed. If so, a new sample is brought into view and processing continues with step 720. Otherwise, image processing is complete. In a presently preferable embodiment, image data can be stored on a CD ROM using a CD ROM burner, such as CRW4416 made by Yamaha of Japan. However, other mass storage media can also be used.

Fig. 7D illustrates a representative block flow diagram of simplified process steps for processing digitized representations of step 716 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 740, a digitized image input is preprocessed . Preprocessing might include, but is not limited to, such operations as background subtraction, thresholding, smoothing, adoptive filtering, edge enhancements, contrast enhancements, histogram equalization. A particular combination of preprocessing steps can be applied to images in successive steps or in parallel to copies of the image.

A simplified example of a smoothing and background subtraction procedure in a MatLab language is presented in computer code below:

```
function Isubtracted = cmBackgrSubtrl(I,k)
% cmBackgrSubtrl(I,k) - simple flat background (=modal*k)
subtraction
% Y = cmBackgrSubtrl(I, k) - image Y is generated by
```

```
% subtraction (with saturation) of modal pixel value of I
multiplied by k
% DEFAULT - k=1
%
if (nargin == 1)
   k=1;
end
if (size(k)~=1)
   error('cmBackgrSubtr1: parameter k should be a number.
Exiting...');
end

%modpixnum = floor(size(I(:),1)/2);
%sortedval = sort( double(I(:)) );
%modpixel = sortedval(modpixnum);
modpixel = median(double(I(:)));
bg = k*modpixel;

Isubtracted = mmsubm(  uint8(I), uint8(round(ones(
size(I))*k*modpixel )) );
```

An example of a procedure for thresholding in computer code (MatLab) is presented below:

```
function thresh = GetThreshByPerim1(I, M)
% GetThreshByPerim1(I) Finds optimal thresholding value
for image I
% N = GetThreshByPerim1(I) Finds thresholding value N for
image I
% N = GetThreshByPerim1(I, M) - tests threshold values up
to M
% DEFAULT M = maximum pixel value in I
% note that GetThreshByArea is significantly faster
% finds a threshold value that causes the maximal change
in the
```

```
% total perimeter of the objects (Russ ????)
% see Matlab_Auto_threshold1_1-23-99.doc for more details
% Note: works somewhat better on SMOOTH images (i.e.
medfilt2(I, [3 3]) two times

if (nargin == 0)
   error (strcat( mfilename,' : at least one parameter
required'));
elseif (nargin == 1)
   M = double(max(I(:)));      %test thresholds up to
maximum pixel value in I
elseif (nargin > 2)
   error (strcat (mfilename,' : too many parameters'));
end

if (size(M)>1)
   error (strcat(mfilename, ' : argument M should be a
number'));
end

Minval = double( min(I(:)));
step = 1;

%generate vertical vector perims with total perimeters of
objects at different
%threshold values
for i=Minval : step : M
     bwI = im2bw(I, i/255);
     prI = bwperim(bwI);
     pr = sum(prI(:));
     if (exist('perims', 'var') == 0) %perims is yet
undefined
          perims = pr;
     else
```

```
            perims = cat(1, perims, pr);
        end
    end

% vector prdiffs contains differences between successive
perimeters
prdiffs = diff(perims);
mindecrease = min(prdiffs);
minvalues = find(prdiffs == mindecrease);
index_of_mindecrease = minvalues(1);
thresh = index_of_mindecrease + 1;

% ==============end GetThresh1=================
```

Thresholding provides a specific intensity, such that pixels darker than the threshold are deemed black, and pixels lighter than the threshold are considered white. The thresholded image can be processed using binary image processing techniques in order to extract regions.

(2). In a step 742+744, the digitized image input is subjected to object identification. This can be accomplished by a variety of procedures, for example by thresholding or edge detection and subsequent morphological opening and closing. Edge detection can be accomplished by means of gradient-based or zero-crossing methods, such as Sobel, Canny, Laplassian, Perwitt, and other methods.

An example of object identification procedure based on Canny edge detection (in MatLab language) is presented below:

```
function Imask = cmMaskDNA1(I);
% cmMaskDNA1 - generates binary mask for cell nuclei
through edge detection
% Imask = cmMaskDNA1(I)
% PARAMETERS
%    I - intensity image (grayscale)
% OUTPUT
%    Imask - BW image with objects from I
```

```
%
% For more details see Notebook Matlab_DNA_masking1_1-22-
99.doc
% Uses SDC Morphology Toolbox V0.7

if (nargin ~= 1)
   error('Wrong number of input parameters');
end
if (nargout ~= 1)
   error('Wrong number of output parameters: one output
argument should be provided');
end


Imask = edge(I, 'canny');
Imask = mmdil(Imask, mmsecross(1));
Imask = mmero ( mmclohole(Imask,mmsecross(1)));
Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
Imask = medfilt2(Imask, [5 5]);

%=========end cmMaskDNA1
=================================
```

However, embodiments can also use other techniques, such as Fast Fourier Transforms (FFT) and the like as known in the art without departing from the scope of the present invention.

(3). In a step 746, a plurality of region features can be determined. For example, in a representative embodiment, image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Features not directly related to individual objects are also being extracted.

An example of a procedure for extraction of some of the features (MatLab language) is presented below:

```
function OData = cmGetObjectsData(I, Ilabel)
% cmGetObjectsData returns array measurements of objects
in image "I" masked by "Ilabel"
% EV 2-3-99; 2-10-99
% OData = cmGetObjectsData(I, Ilabel) returns an array of
morphological and intensity measurements
%    taken from a grayscale image "I". Objects are
identified on a mask image Ilabel, usually
%    created by bwlabel()
% OUTPUT:
% Each row in the output array OData represents
individual object
% columns contain the following measurements:
%
%    1 - Index ("number" of an object);      8  -
Solidity;
%    2 - X coordinate of the center of mass; 9  - Extent;
%    3 - Y coordinate          -"-          ; 10 - Total
Intensity;
%    4 - Total Area (in pixels);              11 - Avg.
Intensity;
%    5 - Ratio of MajorAxis/MinorAxis;        12 - Median
Intensity;
%    6 - Eccentricity;                    13 - Intensity of
20% bright pixel
%    7 - EquivDiameter;                   14 - Intensity of
80% bright pixel
%
% For details on morphological parameters see information
on MatLab imfeature();
```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

if (nargin ~= 2)
   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
   error ('function has 1 output argument (array X by
14)');
end

% finished checking arguments

% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
'MajorAxisLength',...
   'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
...
   'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
for k=1:numobjects
     OData(k, 1) = k;
     OData(k, 2) = ImStats(k).Centroid(1);
     OData(k, 3) = ImStats(k).Centroid(2);
     OData(k, 4) = ImStats(k).Area;
```

```
        OData(k, 5) = (ImStats(k).MajorAxisLength) /
(ImStats(k).MinorAxisLength);
        OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
        OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

        % now collect and assign intensity parameters from
image I

        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
number of pixels in the object
        object_intensities = double(I(object_pixels)); %
need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
will need to get median, 20% and 80% pixels
        total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
        median_intensity = sorted_intensities( floor(
object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
) ;

        OData(k, 10) = total_intensity;
        OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
        OData(k, 13) = pix20; %brightest pixel among dimmest
20%
        OData(k, 14) = pix80; %dimmest pixel among brightest
20%
end %for
```

```
%=================== end function
cmGetObjectsData()==================================
```

(4). In a step 748, quantitative descriptors, characterizing cell state are calculated based on the feature measurements extracted at step 746. For example, histogram distribution of intensities of cell nuclei provides information about the population cell cycle stages.

In a particular embodiment according to the present invention, data analysis techniques for describing the fluorescence patterns of cell portions in multiple cell lines in the presence and absence of compounds are provided. Automated image analysis techniques can include determining one or more regions from around nuclei, individual cells, organelles, and the like, called "objects" using a thresholding function. Objects that reside on the edge of an image can be included or excluded in various embodiments. An average population information about an object can be determined and recorded into a database, which can comprise a database text file or Excel spreadsheet, for example. However, embodiments can use any recording means without departing from the scope of the present invention. Values measured can be compared to the visual image. One or more types of numerical descriptors can be generated from the values. For example, descriptors such as a number of objects, an average, a standard deviation of objects, a histogram (number or percentage of objects per bin, average, standard deviation), and the like can be determined.

In a particular embodiment according to the present invention, data can be analyzed using morphometric values derived from any of a plurality of techniques commonly known in the art. For example, a software package called MetaMorph Imaging System, provided by Universal Imaging Corporation, a company with headquarters in West Chester, PA and NIH Image, provided by Scion Corporation, a company with headquarters in Frederick, Maryland.

Fluorescent images can be described by numerical values, such as for example, an area, a fluorescence intensity, a population count, a radial dispersion, a perimeter, a length, and the like. Further, other values can be derived from such measurements. For example, a shape factor can be derived according to a relationship

4π * area / perimeter. Other values can be used in various embodiments according to the present invention. Such values can be analyzed as average values and frequency distributions from a population of individual cells.

In a particular embodiment according to the present invention, techniques for the automatic identification of mitotic cells are provided. Image analysis techniques employing techniques such as multidimensional representations, frequency-based representations, multidimensional cluster analysis techniques and the like can be included in various embodiments without departing from the scope of the present invention. Techniques for performing such analyses are known in the art and include those embodied in MatLab software, produced by MathWorks, a company with headquarters in Natick, MA.

Scalar values providing efficacious descriptors of cell images can be identified using the techniques of the present invention to perform predictive analysis of drug behavior. In a presently preferred embodiment, a plurality of heterogeneous scalar values can be combined to provide descriptors for each manipulation. By applying predictive analysis routines to the collections of these descriptors, predictive information about any number of manipulations and cell interactions can be extracted.

Fig. 7E illustrates a representative block flow diagram of simplified process steps for analyzing image feature values to obtain descriptors of cell state of step 718 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7E illustrates an input data of descriptors of known manipulations 319. A step 320 of reformatting and transforming data 319 to formats suitable for analysis is performed. Additionally, a "cleaning" process can eliminate outlying data points and the like in the data. Then, in a step 322, a decision is made whether to continue with step 324 or with step 326 based upon determining a particular type of analysis appropriate for the present application or particular type of prediction. If decisional step 322 determines processing should continue with step 324, then, in that step, an error estimate using a set of test descriptors is performed to estimate the quality of a prediction and processing continues with step 320. Once an optimal prediction is achieved, processing continues with step 326. In step 326, optimal transformation parameters and prediction methods are selected for use in

steps 328 and 330 which analyze data about an unknown manipulation. In a step 328, a solution is generated based upon any of techniques including training a neural network, solving a mathematical equation, applying decision tree rules and/or the like. In a step 330, an input data set of unknown descriptors 318 is reformatted and transformed based upon the optimal transformation parameters selected in step 326 using the transformation procedures in steps 320, 322 and 324. In a step 332, predictions techniques are applied to the reformatted manipulations from step 330 and the solution generated in step 328 and a plurality of properties of known manipulations 317 (e.g., therapeutic properties, and the like) in order to determine a prediction of properties of unknown manipulation 316.

Fig. 7F illustrates a representative block flow diagram of simplified process steps for a method of mapping a manipulation of cells to a physiological characteristic in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 750, a plurality of cells, e.g., dead, live, cell fractions or mixtures of cells are provided.

(2) Then, in a step 752, the plurality of cells is manipulated, where manipulation occurs using a source(s) from one or a combination selected from an electromagnetic, electrical, chemical, thermal, gravitational , nuclear , temporal , or a biological source.

(3) Next, in a step 754, a feature value is captured from the plurality of cells. The feature value can include one or any combination of characteristics such as cell count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface area, average intensity, total intensity, and optical density. This list is not meant to be limiting.

(4) Then, in a step 756, a degree of presence of one or more feature values is assigned for each manipulation.

(5) In a step 758, the feature values from the plurality of cells are stored in memory locations. From the memory locations the values can be used for

statistical analyses to produce predictive information about the relatedness of the descriptors of the manipulations to one another. This information is used to infer properties of the manipulations.

Fig. 7G illustrates a representative block flow diagram of a simplified process steps for a method for populating a database with manipulated biological cell information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 760, a plurality of cells in various stages of the cell cycle, A montage image that was used as a source to generate data in Appendix A is presented in Fig. 12., such as for example, the stages of interphase, prophase, metaphase, anaphase, and telophase are provided.

(2) Then, in a step 762, each of the cells in the various stages of mitotic development is manipulated.

(3) Next, in a step 764, an image of the plurality of manipulated cells is captured using image acquisition techniques in order to provide a morphometric characteristic of each of the manipulated cells.

(4) As a preferable option, in a step 766, an image database may be populated with the image of the plurality of manipulated cells.

(5) Following step 764 or optional step 766, a morphological value is calculated from the image in a step 768.

(6) In a step 770, the database is populated with the morphological value.

Fig. 7H illustrates a representative block flow diagram of simplified process steps for a method for populating a database with manipulated biological information, e.g., image acquisition parameters, image feature summary information, and well experimental parameters in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7H illustrates a step 780 in which cells are placed into site on a plate and a manipulation is applied. Then, in a step 781 an image is taken of the cells. In step 782, the image is transferred to an image archive

database. Then, in a step 783, well experimental parameters are entered into the database 787. Well experimental parameters can include cell type, manipulation and the like. In a step 784, image acquisition parameters are transferred to database 787. Image acquisition parameters can include file name, fluorophores and the like. In a step 785, the image acquired in step 781 is analyzed. Then, in step 786, an image feature summary from the analysis step 785 is transferred to database 787.

In step 788, a lookup table for all analyses is provided to database 787. The lookup table provides information about the analyses. In a step 789, a query of database 787 for process data is performed. The results are reformatted. Then in a step 790, the database 787 is queried. Next, in a step 791, features of the manipulations stored in the database are combined and reduced. Next, in a step 793, reduced features of step 791 can be compared. In a step 792, the results of step 793 are recorded in database 787. Then, in a step 794, a report of predictions based on comparisons performed in step 793 is generated.

Fig. 7I illustrates a representative block flow diagram of simplified process steps for acquiring images of manipulated biological information, e.g., cells, cell tissues, and cell substituents in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7I illustrates a step 770 in which a user sets up an image analysis procedure. Then, in a step 772, an image is read into image analysis software. Next, in a step 774, patterns and objects are identified in the image using one or more algorithms. Next, in a step 776, sets of features are extracted from the image. Then, in a step 778, feature information, descriptor values and the like are exported to the database, such as database 787 of Fig. 7H, for recording. Next, in a decisional step 779, a determination is made whether any more images should be taken. If this is so, processing continues with step 772. Otherwise, image acquisition processing is completed.

Fig. 7J illustrates a representative block flow diagram of simplified process steps for populating, acquiring and analyzing images of manipulated biological information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,

modifications, and alternatives. Fig. 7J illustrates a step 300 of placing a plate onto an imaging stage and reading a bar code. Then, in a step 301 an autofocus procedure is performed. Next, in a step 302, a first optical filter configuration is selected and an image is collected. Then, in a decisional step 303, a determination is made whether more than one image per optical configuration can be taken. If so, then, in a step 304, a new position within the well is targeted and another image is collected. Then, in a decisional step 305, a determination is made whether any more images need to be collected. If this is so, step 304 is repeated until all images for a particular well have been collected. After one or more images are collected for the well, in a step 306, the stage is returned to a starting position within the well, and a montage is created from collected images. The results are named with a unique file name and stored.

In a decisional step 307, a determination is made whether any more optical channels in the well can be imaged. If this is so, then in a step 308 the next optical filter configuration is selected and an image is collected. Processing then continues with decisional step 303, as described above. Otherwise, if no further optical channels in the well can be imaged, then in a decisional step 309 a determination is made whether any wells remain to be imaged. If not all wells have been imaged, then in a step 310, the stage moves to the next well and processing continues with step 301, as described above. Otherwise, if all wells on the plate have been imaged, then in a decisional step 311, a determination is made whether any more plates can be processed. If this is so, then processing continues with step 300 as described above. Otherwise, in a step 312, the information is stored to a CD or other storage device as a backup.

Fig. 7K illustrates a representative block flow diagram of simplified process steps compound based upon information about effects of one or more known compounds on a cell population in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7K illustrates a step 340 of populating a database with descriptors for known compounds. Such descriptors can be determined from imaging the cell population. However, in some embodiments, descriptors can be derived by measurements and combinations of measurements and the like. Then, in a step 342, descriptors for the unknown compound are determined from imaging a

second cell population. The second cell population has been treated with the unknown compound. Then, in a step 344, a relationship between the descriptors determined from the unknown compound with the descriptors determined from the known compounds can be determined. Finally, in a step 346, an inference can be made about the unknown compound based upon the descriptors of the known compounds from the relationship determined in step 344.

Accordingly, the present invention provides a novel database design. In a particular embodiment according to the present invention, a method for providing a database comprises measurement of a potentially large number of features of one or more sub-cellular morphometric markers. Markers can be from any of a large variety of normal and transformed cell lines from sources such as for example, human beings, fungi, or other species. The markers can be chosen to cover many areas of cell biology, such as, for example markers comprising the cytoskeleton of a cell. The cytoskeleton is one of a plurality of components that determine a cell's architecture, or "cytoarchitecture". A cytoarchitecture comprises structures that can mediate most cellular processes, such as cell growth and division, for example. Because the cytoskeleton is a dynamic structure, it provides a constant indication of the processes occurring within the cell. The cytoarchitecture of a cell can be quantified to produce a one or more scalar values corresponding to many possible cellular markers, such as cytoskeleton, organelles, signaling molecules, adhesion molecules and the like. Such quantification can be performed in the presence and absence of drugs, peptides, proteins, anti-sense oligonucleotides, antibodies, genetic alterations and the like. Scalar values obtained from such quantification can provide information about the shape and metabolic state of the cell.

In a presently preferred embodiment, scalar values can comprise morphometric, frequency, multi-dimensional parameters and the like, extracted from one or more fluorescence images taken from a number of cellular markers from a population of cells. Two or more such scalar values extracted from a plurality of cell lines and markers grown in the same condition together comprise a unique "fingerprint" or descriptor that can be incorporated into a database. Such cellular descriptors will change in the presence of drugs, peptides, proteins, antisense oligonucleotides, antibodies or genetic alterations. Such changes can be sufficiently unique to permit a correlation to be drawn between similar descriptors. Such

correlations can predict similar properties or characteristics with regard to mechanism of action, toxicity, animal model effectiveness, clinical trial effectiveness, patient responses and the like. In a presently preferred embodiment, a database can be built from a plurality of such descriptors from different cell lines, cellular markers, and compounds having known mechanisms of action (or structure, or gene response, or toxicity).

The present invention also provides database and descriptor comparisons according to other embodiments. In a particular embodiment according to the present invention, measurement of scalar values or features can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be compared using a plurality of techniques, such as a technique of creating "phylogenetic trees" of a statistical similarity between the descriptors from various drugs. In a present embodiment, scalar, numeric values can be converted into a nucleotide or amino acid letter. Once converted into a corresponding nucleotide representation, the descriptors can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. In an alternative embodiment, numeric values for the fingerprints can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound fingerprint with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphometric properties and features of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, a clustering can be performed on acquired image descriptors. Some embodiments can comprise statistical and neural

network - based approaches to perform clustering and comparisons of various descriptors. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data. In some embodiments, clustering and comparing can be performed on features extracted from cell images. In a presently preferable embodiment, procedures for comparisons and phylogenetic analysis of biological sequences can be applied to data obtained from imaging cells.

Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the program Phylip, available at http://evolution.genetics.washington.edu/phylip.html, and other packages listed at http://evolution.genetics.washington.edu/phylip/software.html . However, select embodiments according to the present invention can comprise a technique of statistical classification, statistical clustering, distance based clustering, linear and non-linear regression analysis, self-organizing networks, and rule-based classification.

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes. Resulting "pseudo-sequences" can be subjected to analysis by a sequence comparison and clustering program.

Other types of databases can also be provided according to other embodiments. The database includes details about the properties of a plurality of standard drugs. When the descriptor of a test compound is compared to the database, predictions about the properties of the test compound can be made using any known property of the other compounds in the database. For example, properties about a compound in the database could include structure, mechanism of action, clinical side effects, toxicity, specificity, gene expression, affinity, pharmacokinetics, and the like. The descriptor of a compound of unknown structure from a natural products library could be compared to the descriptors of compounds with known structure and the structure could be deduced from such a comparison. Similarly, such information could lead to better approaches to drug discovery research including target validation

and compound analogizing, as well as pre-clinical animal modeling, clinical trial design, side effects, dose escalation, patient population and the like.

According to the present invention, databases can be integrated with and complementary to existing genomic databases. Differential genomic expression strategies can be used for drug discovery using database technology. In one particular embodiment, cell data and cellular response data can be associated with a genetic expression profile assay to form a single assay. Live cells expressing fluorescence markers can be treated with a drug, imaged and analyzed for morphometry; and then analyzed for mRNA for expression. Such embodiments can provide rapid development of tools to link cellular behavior with functional genomics.

Database methods according to the present invention can be used to predict gene function and to assist in target validation. Databases that include genetic diversity, i.e., having cellular descriptors from cells of differing genetic backgrounds (tumor, tissue specific, and gene knock out cell lines), can provide the capability to compare cells of unknown genetic background to those in the database. Similarly, the descriptor of an unknown cellular portion in the presence of multiple drugs can be queried against the descriptors of the known markers in the database. For example, if an unknown gene is tagged with Green Fluorescent Protein (GFP), the database may be used to identify the cellular portions for which that unknown gene encodes.

According to the present invention, target validation and specialized cell-based assay screening can be performed using database systems and methods to serve as a universal high-throughput cell-based assay that can evaluate the molecular mechanism of drug action. As new genes are isolated and identified, a large collection of available gene-based knowledge is becoming available. From this large collection of new genes, potential protein targets can be identified using the genomic tools of sequence analysis and expression profiling. However, unless a gene mutation is tightly linked to a disease state, further validation of individual targets is a time consuming process, becoming a bottleneck in drug discovery. Furthermore, robotics and miniaturization are making "High Throughput Screening (HTS)" the industry standard, substantially reducing the time and cost of running a target-based biochemical assay. Therefore, it is now possible to routinely screen large libraries and use a resulting "hit" to validate the target. In such approaches, a specialized cell-based assay would be developed to test hits for each target. Since this often involves

the creation of cell lines expressing new markers, this stage may also become a bottleneck that cannot keep pace with HTS. In addition, these cell-based assays may not be amenable to high-throughput screening, making it difficult to test the increasing number of analogs arising from combinatorial chemistry.

In a particular embodiment according to the invention, a rapid characterization of large compound libraries for potential use as pharmaceutical products can be provided by predicting properties of compounds that relate to the compounds' potential as bioactive drugs. In many drug discovery situations, virtually millions of compounds can be passed through a HTS assay against a small number of validated targets. These assays produce hundreds to thousands of potential hits. These hits can then be subsequently screened by a pipeline of secondary and tertiary screens to further characterize their specificity, often time completely missing non-specific interactions with other proteins. Techniques according to the present invention can provide a replacement to such screening operations by providing information about cellular accessibility and mechanism of action for the hits coming from a HTS system. Furthermore, it can replace the biochemical HTS assay and allow rapid and accurate identification of attractive compounds from large libraries without an intervening biochemical assay. The cell information can be predictive of whether to continue into an animal model for each compound, and which animal model to pursue.

The principles of the present specifically contemplate a wide variety of research methodologies, or usage scenarios, implementing these principles. The following discussion of three such scenarios is by way of illustration and not limitation. Study of the principles enumerated herein will render evident to those skilled in the art certain additional methodologies or usage scenarios enabled by the teachings hereof. The present invention specifically contemplates all such modifications. The following description presents some specific embodiments and scenarios that represent a broader use of cellular phenotypic data and characterizations to deduce mechanisms of action and other features of cellular responses to various stimuli. Such procedures generally involve producing a quantitative cellular phenotype based upon two or more cellular attributes and then comparing that phenotype to phenotypes previously stored and indexed. Such

procedures make use of databases or other repositories of biological information. The invention is not limited to the specific embodiments described here.

Considering first the procedure 2000 depicted in Figure 20, a compound has been identified as having a particular cellular activity. See 2004. For example, a compound may be found to inhibit the growth of certain cancer cell *in vitro* by a specific and desired mechanism of action. This may be a particular company's "gold standard."

Next, the compound is analyzed at 2006 in terms of its effect on one or more cell lines. More specifically, the compound is linked, virtually, to a particular phenotype. Two or more values or measures of cellular attributes characterize that phenotype. These attributes are quantified in the context of specific cellular markers.

In one example, the cellular marker is an organelle such as a nucleus or Golgi apparatus. Measured attributes useful for characterizing an associated phenotype include geometric parameters (e.g., size, shape, and/or location of the organelle) and composition (e.g., concentration of particular biomolecules within the organelle).

The phenotype may be characterized by administering the compound of interest to various cell lines and in various concentrations. In each example within this matrix, the attributes of interest are measured. Ultimately, certain phenotypic features (combinations of attribute values) are associated with the compound of interest. These features provide a template for the phenotype.

Next, using the phenotype as identified at 2006, the process identifies other compounds providing similar features. The goal here is to present a list of compounds having a mechanism of action similar to that of the compound that started the process. This allows researchers to identify a mechanism of action, if not already known, for their compound and to draw conclusions based upon their compound's link to other known compounds (which may not be chemically/structurally similar to the compound of interest).

Identifying similar compounds based upon phenotype can take many paths. Most will involve some mathematical basis. For example, the phenotype defined at 2006 can be represented as a fingerprint or vector comprised of multiple scalar values of cellular attributes (as described above). The phenotype representation can then be compared against known phenotypes characterized by the same format

(e.g., they are all characterized as vectors having the same attribute set, but with different values of the attributes). The comparison may be as simple as a Euclidean distance or more sophisticated as a neural network or multivariate statistical correlation.

The known compounds and associated phenotypes may be stored as database records or other data structures that can be queried or otherwise accessed as part of the identification procedure. The compounds may also be associated with other relevant data such as clinical toxicity, cellular toxicity, hypersensitivity, mechanism of action, etc. (when available).

Compounds found to be sufficiently similar to the starting compound are returned for consideration by researchers. A data processing system may rank such compounds based on degree of similarity to the starting compound. In some cases, the system may even provide similarity scores associated with the listed compounds.

Often researchers wish to determine whether their particular compound has clinical or biochemical effects beyond those that they are already aware of. In a typical scenario, the compound of interest was selected based upon its strong binding a target or its stimulation or inhibition of cell growth in a particular cell line. The process associated with 2010 has likely identified the compound of interest as having a particular mechanism of action based on phenotypic similarity to other compounds having a similar mechanism of action. However, within the region of biochemical space, there may be subspaces (characterized by subphenotypes) that correspond to separate properties. For example, within the phenotypic space associated with one mechanism of action, there may be subspaces associated with clinical toxicity, cellular toxicity (likely overlapping the clinical toxicity space), and little or no toxicity. Obviously, a researcher would like to know whether her compound is likely to be toxic.

Thus, the process 2000 may include characterizing the compound of interest in terms of its distance from (i.e., similarity to) specific phenotypes having known characteristics. In a typical example, the known characteristic is toxicity. This feature allows the researcher to quantify her compound in terms of mechanism of action AND toxicity (or in terms of two or more other relevant properties associated

with phenotype). To allow simple ranking or characterization, compounds of interest may be scored according to a simple or weighted Boolean expression.

A second scenario of interest is depicted in Figure 21. This scenario again defines a phenotype in terms of a quantifiable vector or other measure. However, rather than using a compound of interest to generate the phenotype, some other cellular stimulus is used to generate the phenotype.

As shown, a process 2100 begins with receipt of cells of interest. See 2104. In many situations, the cells are produced by a genetic or epigenetic process that affects the expression level or activity of a particular protein. More generally, any cellular stimulus (e.g., radiation level and type, gravity level, magnetic field, acoustic perturbations, etc.) can be used to generate the cell line of interest. Importantly, this stimulus affects the phenotype and can be correlated therewith.

In the context of drug discovery, a gene encoding for a particular target can be genetically knocked out, underexpressed, overexpressed, expressed in a non-native state, etc. This may be accomplished via standard procedures involving genomic modification, translation or transcription apparatus modification (e.g., use of antisense nucleic acids), blocking target activity (using antibodies to a receptor site for example), and the like. These processes will generally affect the phenotype in some quantifiable way. Importantly, they clearly and unambiguously define a cellular phenotype associated with altering the activity of the target protein.

At 2106, the process involves measuring one or more cellular features from the cell line of interest to define/quantify the phenotype. This may be accomplished as described above with reference to 2006. Next, at 2108, the cellular phenotype generated in this manner is used to identify and rank a set of compounds associated with the phenotype. This operation may proceed in the manner of operations 2008 and/or 2010 from Figure 20.

Finally, at 2110, the process clusters the compounds returned at 2108 by a mechanism of action. The operation 2106 has tightly bound a mechanism of action to a phenotype. Various compounds characterized and stored in a system database may be tentatively assigned a mechanism of action or may have no suggested mechanism of action. By matching their virtual phenotype to the phenotype generated at 2106, one can create or strengthen an association between the compounds and mechanism of action relevant to the stimulus at 2104.

Considering now Figure 22, a third scenario is depicted. This scenario again involves using a virtual phenotype to glean information relevant to a mechanism of action or other cellular activity. In this case, assay data from a group of compounds (e.g., a primary or focused library) is used to elucidate a phenotype.

As shown, a process 2200 begins by identifying a target protein. See 2204. Then, at 2206, the process involves identifying positive and negative biochemical hits. More generally, this may involve ranking a number of compounds based upon their interaction with the target. In a specific case, the compounds are ranked based upon their binding affinities to or ability to inhibit the enzymatic activity of the target protein.

After the compounds have been characterized in some manner based upon their interaction with the target, they are used to define a cellular phenotype. See 2208. Generally, the techniques to accomplish are the same as described with reference to operation 2006 of Figure 20. In this case however, one may obtain a strong correlation between mechanism of action (involving the target) and phenotype by using multiple of the compounds identified at 2206. For example, some of the "best hits" may be administered to cell lines in various concentrations. And some of the least effective compounds may also be administered. Cellular attributes that are more strongly exhibited with increasing concentration of the best hits (and not exhibited or exhibited only weakly upon administration of the negative hits) can be used to define the virtual phenotype. In a related approach, compounds having widely varying levels interaction with the target are administered to cells. Those cellular attributes that vary linearly or at least monotonically with the degree of interaction between the target and compound represent attributes that can be used to define the virtual phenotype.

After the cellular phenotype has been defined, previously characterized compounds may be clustered with that phenotype. See 2210. As with operation 2110 of Figure 2, this may create or strengthen an association between a mechanism of action and various compounds in a database.

Finally, and optionally, procedure 2200 may provide a "higher resolution" mechanism of action for the compounds identified at 2206. See 2212. Presumably interaction with the target suggests a specific mechanism of action or at least some aspect of a mechanism of action. However, a given target may participate

in a larger cellular mechanism of action – unknown to researchers. Further, a compound may that binds with the target may participate in multiple mechanisms of action – some of which do not involve the target. By linking the target (and its positive hits) to a particular phenotype, some of these additional cellular level activities can be elucidated. The defined phenotype may have been previously identified as associated with other mechanisms of action or higher resolution mechanisms of action. Thus, the phenotype identified at 2208 can be leveraged to generate a higher resolution mechanism of action at 2212.

As suggested in the above discussion, compounds and associated phenotypes may be stored as database records. Such databases can take on many flavors. In one example, a database includes various pieces of information relevant to oncology. Such database may include numerous compounds classified by cellular phenotype, mechanism of action, toxicity, etc. More specifically, the database may include data on commercially available compounds clustered by cellular phenotypes corresponding to mechanisms of action. Further the databases of interest may extended or combined (via standard relational tables and algebra for example) to include additional data such as pharmacology data, cellular genomics data, gene expression data, protein expression data, etc. In a specific example, the database includes measurements made on a subset of the NCI60 cell lines, using DNA, Golgi apparatus, and/or microtubules as markers for defining the phenotypes. Other data includes dosage response information, variation in effect over time, etc. The compounds populating the database could include known National Cancer Institute oncology study compounds. In a specific embodiment, the compound set includes some or all of the compounds mentioned in the article "A gene expression database for the molecular pharmacology of cancer," Nature Genetics, 24, pp. 236-244 (March 2000).

Various biological analyses may be conducted to develop additional information for characterizing compound mechanisms of action, etc. For example, a cell count analysis may be used to develop dose response curves, GI 50 data, etc. The cell cycle may also be analyzed to find out how various stages in the cycle vary in response to particular stimuli. The Golgi apparatus may be analyzed to determine whether it is in a normal state, a dispersed state, a diffused state, etc. As another example, tubulin may be analyzed to determine whether it is normal, de-polymerized,

over-polymerized, bundled, etc. Obviously, combinations of such analyses may be performed. For example, properties of the Golgi apparatus or tubulin may be analyzed over one or more cell cycles.

In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs, such as clinical trial and patient response information, will be used in a similar fashion as the pre-clinical information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions will be able to provide predictive value for this aspect of drug development.

Although the above has generally been described in terms of specific hardware, software, and methods, it is understood that many alternatives can exist. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives. Some examples according to the present invention are provided below.

EXPERIMENTS

To prove the principle and demonstrate the objects of the present invention, experiments have been performed to determine the effects of manipulations on cell structure using imaging and analysis techniques applied to a variety of situations. These experiments were performed by growing multiple cell lines in the presence of multiple compounds, or substances. Cells were fixed and stained with fluorescent antibodies or labels to multiple cellular portions . One or more images of the cells were then obtained using a digital camera. Descriptors were built by quantifying and/or qualifying patterns of one or more feature from each image in the cell lines under study. A database was built from the descriptors. As the database grows, it should be able to predict the mechanism of action of an unknown drug by comparing its effect with the effects of known compounds or to identify data clusters within large libraries of compounds.

In a first experiment, an automated method to count the number of cells and differentiate normal, mitotic, and apoptotic cells was created. Approximately, 5,000 HeLa cells were plated per well in a 96 well plate and grown for 3.5 days. The cells were fixed with –20° MEOH for 5 minutes, washed with TBS for 15 minutes, and then incubated in 5 mg/ml Hoechst 33342 in TBS for 15 minutes. Then, 72 images were collected with a 40x objective and 75 ms exposure time.

The analysis was performed on objects that met a certain size criteria that was based on 1) measuring the size of objects in the image that were clearly not cells and 2) excluding the first peak of the area histogram (Fig. 8B values 1-4654).

Histograms of the individual object data were generated for each type of feature. Fig. 8A shows the histogram for average intensity, and Fig. 8B shows histogram data for the area of each object. Fig. 8C shows the scatter plot of the average intensity vs. the area of all of the objects. The pattern of the scatter plot showed an interesting pattern: a large cluster of cells in one region of the graph, with a scattering of object points in other regions. Because mitotic structures are identified as particularly bright objects, most likely due to the biological fact that the chromatin is condensed, the original Hoechst images could be used to identify which cells were either undergoing mitosis, or otherwise looked abnormal. Manual inspection of 917 cells resulted in the classification of each object. Fig. 8D shows a graph where each type of cellular classification is delimited. This graph clearly shows that the mitotic nuclei are brighter than the interphase nuclei. Further, the different phases of the cell cycle can be separated using these two features. Figs. 8E-8F show bar graphs of the average and standard deviations of the areas and average intensities for each cell classification type. These graphs show that interphase nuclei are statistically less bright than mitotic nuclei and that telophase nuclei are statistically smaller than other mitotic nuclei.

Each image was thresholded to an intensity level of 20. A standard area value was set at 9500 pixels. Automated information gathering about all of the objects was done and collected into an Excel spreadsheet (for more information see, section on imaging system). The following information was recorded:

IMAGE NAME
OBJECT #

AREA
STANDARD AREA COUNT
PERIMETER
FIBER LENGTH
FIBER BREADTH
SHAPE FACTOR
ELL. FORM FACTOR
INNER RADIUS
OUTER RADIUS
MEAN RADIUS
AVERAGE INTENSITY
TOTAL INTENSITY
OPTICAL DENSITY
RADIAL DISPERSION
TEXTURE DIFFERENCE MOMENT
EFA HARMONIC 2, SEMI-MAJOR AXIS
EFA HARMONIC 2, SEMI-MINOR AXIS
EFA HARMONIC 2, SEMI-MAJOR AXIS
ANGLE
EFA HARMONIC 2, ELLIPSE AREA
EFA HARMONIC 2, AXIAL RATIO
EFA HARMONIC 3, SEMI-MINOR AXIS

The following results were obtained:

- 1,250 objects were counted
- 201 of those objects has standard area counts > 2 (area > 19000 pixels)
- 195 objects had areas < 6000 pixels
- 1529 objects estimated in total
- 1328 object areas are > 6000 pixels
- The data was reduced to 917 objects that were 6000 < area < 19000
- For the 917 objects a scatter plot of area vs. average intensity and a histogram of the average intensity were generated.

- 116 objects that had average intensity intensities > 60 were manually looked at to determine their morphology.
- Of those 116 objects:

  6 were dead or indistinguishable

  4 were interphase

  30 were prophase

  32 were metaphase

  24 were anaphase

  20 were telophase (10 pairs)

- 12 prophase objects were missed because of gray scale cut off. (8 of those prophase cells had gray scale values > 57, as did 7 interphase)
- 1 telophase object was missed because it was too small (<6000)
- 1 prophase object was missed because it was too big (>1900)
- 16 mitotic objects were missed because they were parts of objects with standard count > 2.

In sum, out of 917 single objects, the analysis correctly identified 106 out of 130 mitotic objects, or (81% predictive, 91% of identified mitotics). Out of 917 single objects, the analysis incorrectly identified only 10 non-mitotics as mitotics (1% total, 8% of identified mitotics); 14 mitotics as interphase (1.4% total, 1% interphase). An automated classification system that would automatically assign values to each object using these or other measurement features can thus be developed, utilizing the principles set forth herein.

In a second experiment, the effects of Taxol on MDCK cells and the different types of morphological effects were observed. A plurality of MDCK cells grown in 96 well plates were treated with Taxol for 4.5 hours at different concentrations (10 uM-1pM). They were then fixed, labeled with Hoechst, and imaged.

This experiment used a labeling protocol comprising: MEOH fix at –20°, Wash in PBS, Block in PBS/BSA/Serum/Triton-X 100, Incubate with 5 μg/ml Hoechst 10 minutes, and wash.

Cells were inspected for different morphologies and manually counted at each different drug concentration in one well. Fig. 9 shows example images from each drug concentration and the different types of morphologies and cells are highlighted. Fig. 10 shows the distribution of each morphology within the cell population as a function of drug concentration. The higher the concentration of Taxol, the larger proportion of cells underwent apoptosis, and the fewer number of normal mitotic cells were detected.

In a third experiment, the purpose was to determine whether the automated analysis methods developed in the first experiment can detect differences in Hoechst morphology in the presence of 6 known compounds at one concentration and exposure time in one cell line. In this experiment, HeLa cells were separately treated with 6 compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black-walled 96 well tissue culture treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of cytochalasin D (CD), Taxol, hydroxyurea, vinblastine, nocodazole, and staurosporine was added to different wells at a 1:100 addition in DMSO.

The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. Then, 9 images per well were collected of the Hoechst staining using a 10x objective.

The low magnification images taken of Hoechst were run through the automated image analysis method described in the first experiment. Plots of the average intensity and area were made of each compound. Fig. 11 shows the scatter plots of the compounds. The scatter plots of each compound are visually distinct. For example, cells treated with CD are smaller than control, and cells treated with Hydroxyurea are larger and brighter. Furthermore, the number of cells per well was very different (data not shown).

The effects of different compounds can be clearly and automatically distinguished by identifying changes in cellular morphology. This method can also be used to count adherent cells.

The next experiment was to develop clustering algorithms that assign statistically meaningful values to the representative two dimensional data shown in Fig. 10, and even more complicated clustering of all of the multidimensional data that can be extracted across one, and multiple images.

A fourth experiment was performed to obtain high magnification images of two markers in the presence of drugs. In this experiment, HeLa cells were treated with 80 generic compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black walled 96 well tissue culture-treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of each compound from the Killer Plate from Microsource Discovery Systems (Gaylordsville, CT) was added to different wells at a 1:100 addition in DMSO. The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. In addition to being labeled with Hoechst 33342 (against chromatin), cells were also labeled with 1 unit of rhodamine-conjugated phalloidin (against actin) for 30 minutes.

The 96 well plate was imaged twice. Once, 9 images per well were collected of the Hoechst staining using a 10x objective. After this, one image per well of both the phalloidin and Hoechst staining was collected using a 40x objective.

The resulting high magnification images were analyzed qualitatively and distinct pattern differences were detected in both the Hoechst and phalloidin images. Fig. 12 shows three example images from the experiment. The top row is the Hoechst staining, and the bottom row is the phalloidin staining from the same well. The columns show the images from wells treated with just DMSO (control), cytochalasin D, and Colchicine. The morphology of each marker is different in the presence of each drug. Interestingly, there is an effect in the morphology of the chromatin in the Hoechst image of cytochalasin D, which directly targets the actin cytoskeleton (and thus there is an expected effect in the phalloidin image). Also, there is an effect on the actin cytoskeleton, compared to control, in the presence of colchicine that directly targets the microtubule network.

The low magnification images were analyzed as described in the first experiment, and different patterns were seen in both the average intensity vs. area plots, and in the number of cells per well (data not shown). Thus, changes in patterns of a marker that is "down-stream" from the direct target of a compound are detectable. Automated image analysis protocols for actin and other markers can be developed similarly, again utilizing the principles set forth herein.

A fifth experiment was performed to test quadruple labeling of 9 different cell lines grown in normal conditions. In this experiment, NCI-H460, A549, MDA-MD-231, MCF-7, SK-OV-3, OVCAR-3, A498, U-2 OS, and HeLa cells were plated. Then, the cells were fixed and stained for portions of the each cell known as DNA, tubulin, actin, and Golgi.

The following table summarizes the procedures for this experiment:

Action	Active Ingredient/Notes	Buffer	Vol/ well	Desired Time	Temp
Remove media	NOTE: gently by pipetting, not aspiration				
Fix	4% Formaldehyde	PBS	100µl	20 min	rt
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt

Permeabliz e	0.1% Triton X-100	TBS	100µl	10 min	rt
Permeabliz e	0.1% Triton X-100	TBS	100µl	10 min	rt
Block	% BSA % Serum Filter sterilize before use	TBS w/azide	100µl	1hr or o/n	rt or 4°C
Primary Antibody	1:1000 dilution of DM1α	TBS + 1% BSA + 0.1% TX-100	50µl	1hr or o/n	rt or 4°C
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt
Fluorescent Stain	FITC lens culinaris 1:500 Rhodamine-Phalloidin 1:500 CY5 goat anti-mouse 1:100	TBS + 1% BSA + 0.1% TX-100	50µl	1 hr.	rt, dark
Wash		PBS	100µl	5 min	rt, dark
Hoechst	1:1000 dilution of 5mg/ml	TBS	100µl	15 min	rt, dark
Wash		PBS	100µl	5 min	rt, dark
Wash		PBS	100µl	5 min	rt, dark
Wash		PBS	100µl	5 min	rt, dark
Store		PBS	200µl	1 month	4°C

Cells were plated out at different densities for 48 hours. Cells were fixed and labeled by the above method. Cells were imaged using an automated imaging system that collected 9 images from each marker using a 10x objective.

Higher magnification images were collected of a few cells for demonstration purposes.

In this experiment, each cell line demonstrated different morphological patterns as determined by phase. For example, A549 cells are much more compacted than OVCAR-3 cells as determined by phase contract imaging (data not shown). The different fluorescent markers showed even bigger differences between different cell lines. Figs. 13 and 14 show 4 panels of each marker for A549 (Fig. 13) and OVCAR-3 cells (Fig. 14). The markers are Hoechst (upper left), Phalloidin (upper right), Lens culinaris (lower left), and DM1a antibody (lower right). The following table summarizes the qualitative differences between these images:

MARKER	A549	OVCAR3
Hoechst/DNA	small	large
Phalloidin/actin	fuzzy	crisp - many stress fibers
Lens culinaris/Golgi	compact	Disperse/punctate
DM1alpha Tubulin	perinuclear	evenly distributed

Higher magnification images were taken of the OVCAR3 cells. Fig. 15 shows the same markers at 20x, and Fig. 16 shows the markers at 40x. While the highest magnification images show the most detail, these images illustrate that very little morphological or feature information is lost in the 10x images.

These data exemplify the differences in morphology seen between different cell types. Thus the automated image analysis software can be customized for each marker in each cell type. Different drugs should effect these morphologies differentially.

An automated quantification method for each marker and cell line can be similarly developed.

A sixth experiment was conducted with a more sophisticated software package and to develop more flexible image recognition algorithms. In this experiment, prototype image features extraction was performed using MatLab programming language with image toolbox and SDC morphology toolboxes. Algorithms are being developed that will automatically identify objects on images and

to measure various morphological and feature parameters of these objects. Many different features for each of the cellular markers were acquired.

An example of a MatLab program called "AnalyseDNA" that takes as an input an unlimited number of images, identifies individual objects in these images based on either their intensities, or based on edge-detection algorithms, and extracts a number of morphological and intensity characteristics of these objects. A copy of this program follows:

Listing of the AnalyseDNA.m program and of some of the supporting subroutines

```
function files_analysed = AnalyseDNA(filemask, outpath,
nx, ny, filter_range, dext, modifier, sfname)
% AnalyseDNA performs measurements on files of DNA images
% V1. EV 2-11-99; 2-15-99; 2-16-99
%
% files_analysed = AnalyseDNA(filemask, outpath, nx, ny,
filter_range, dext, modifier, sfname)
%
% PARAMETERS:
%     ALL PARAMETERS ARE OPTIONAL
%
%     FILEMASK - mask for file names to be analyzed
INCLUDING PATH(for example c:\images\*.tif)
%     DEFAULT '.\*.tif' (all *.tif files in the current
directory).
%
%     OUTPATH - path to a directory where all the output
files will be placed.
%     DEFAULT - output is saved in the same directory
which contains images
%
%     NX, NY - number of individual images in montage
images along X and Y axes (DEFAULT 1)
%
```

```
%     FILTER_RANGE - 3 col-wide array (or[]). Specifies
how data is filtered when summary is calculated
%     this parameter internally is passed to GetDNAData
and then to GetSummaryData - see these
%     functions for details. For example: [2 2 Inf; 6 100
8000] will case all raws of data for which
%             values in column 2 are less than 2 and all raws
where values in column 6 are less than 100 or
%             more than 8000 to be excluded from all
calculations of a summary.
%     DEFAULT - [] (means do not filter, summarize all
data)
%
%     DEXT - string. Extension for data files being saved.
%     DEFAULT 'dat';
%
%     MODIFIER - this modifier is 'SUMMARY', summary file
is created;
%             'SUMMARY ONLY' - only summary is generated,
data for individual files are not saved
%
%     sfname - string. File name of a summary file
%     DEFAULT 'summary[date].dat'
%
% OUTPUT:
%
%     AnalyseDNA works on image files or montages. For
each image file it creates a tab-delimits file of
measured
%     parameters of all the objects in the montage with
the same base name as a montage file and extension
specified
```

```
%     by dext parameter (or .dat by default) and file
'errors[date].err' - with the list of files that matched
the
%     filemask but could not be processed.
%     If 'summary' or 'summary only' modifier is
specified, it also creates a single file
'summary[date].dat' (or
%     different extension, if specified by DEXT) which
contains summary information for all analyzed files.
%
%     ALL OUTPUT FILES are saved in a directory specified
by OUTPATH parameter
%
%     RETURNS *files_analysed* - number of files that have
been successfully processed.
%
%     Column designations in the output files are
described in GetDNAData
%
% FILE NAME CONVENTIONS
%     AnalyseDNA attempts to identify a number for each
file to identify the file in summary output.
%     It does that by looking for the first space or
underscore, followed by a number and then takes
%     as many successive numbers as it can find. If it
fails to identify a number it assigns a
%     default which is -1
%
%
% SEE ALSO GetDNAData, GetSummaryData
%
% TO DO   improve error handling in opening and writing
files (GLOBAL error_file ?)
```

```
%           include procedures for writing text headers
into the output files

if nargin > 8
   error ('Wrong number of input parameters');
end
if nargout >1
   error ('Wrong number of output parameters: only one
allowed');
end

% set defaults
need_summary = 0;
summary_only = 0;
use_default_outpath = 0;
datestring = datestr(floor(now));
if nargin == 7        % set default summary file name
   sfname = ['summary' deblank(datestring)]; % extension
will be appended later based on dext
   if  deblank(upper(modifier)) == 'SUMMARY'
      need_summary = 1;
   elseif deblank(upper(modifier)) == 'SUMMARY ONLY'
      need_summary = 1;
      summary_only = 1;
   else
      error (['Wrong parameter: unknown modifier '
modifier]);
   end
end

if nargin == 5
   % default data file extension
   set dext = 'dat';
end
```

```
if nargin == 4
   % default filter range
   filter_range = [];
end
if nargin == 3
   ny = 1; % default number of images in montage along Y
end
if nargin == 2
   nx = 1;
end
if nargin == 1
   use_default_outpath = 1;
end
if nargin == 0
   filemask = '*.tif'
end

% check parameters
if ( ~ischar(filemask) |  ~ischar(dext) | ~ischar(sfname)
)
        error('Wrong parameter type: filename, filepath,
dext and sfname should be strings');
end
if ( ( size(nx) ~= [1 1] ) | ( size(ny) ~= [1 1] ) )
   error ('Wrong parameter type: nx and ny should be
scalars (1x1 arrays)');
end
if (~isempty(filter_range) & size(filter_range, 2) ~= 3)
   error ('Wrong parameter type: filter range should be
[] or 3 - cols-wide array');
end
% end testing parameters

% Generate list of files to process
```

```
datapath = getpath(filemask);
if use_default_outpath == 1
   outpath = datapath;
end
if exist(outpath, 'dir') ~= 7
   error(['Path ' outpath, 'not found. Exiting..']);
elseif exist(datapath, 'dir') ~= 7
   error(['Path ' datapath, 'not found. Exiting..']);
end

sfname = makefullname(outpath, sfname, dext);
if need_summary == 1
   if exist(sfname, 'file')
      disp(['File ', sfname, 'already exists!']);
      input ('Press ^C to abort, Enter to delete and
continue');
      delete(sfname);
   end
end


flist = FileList(getfname(filemask), datapath);
numfiles = size(flist, 1); % total number of files to
process
disp(['About to process ', num2str(numfiles), ' files']);
%DEBUG  - commented out "input" to run from Wrod
input('Press ^C to abort, Enter to continue');

% main loop where the job gets done:
error_file = makefullname(outpath, ['error' datestring
'.err']);
num_processed = 0;
num_error =0;
```

```
for i = 1:numfiles
     % first generate file name for a data output file
   current_fullname = flist(i, :); % full name with path
and extension
   current_datafile = makefullname(outpath,
makefname(getbasefname(current_fullname), dext) );

   %extract number from a filename
   fnumber = getfilenumber(current_fullname);

   % load an imagefile, record errors
   read_error = 0;
   try
       I = imread(current_fullname);
       %DEBUG
       disp (['Image file #', num2str(fnumber), '
loaded']);
   catch
      % record file-opening error in an error_file
      read_error = 1;
      num_error = num_error +1;
      msg = [current_fullname ': ' lasterr];
      add_error_msg(error_file, msg);
   end

   % extract and write data to a file in outpath
   if read_error ~=1
      if (need_summary == 0)
         %DEBUG
         disp (['Starting analysis of file #',
num2str(fnumber), '.']);
         current_data = GetDNAData(I, nx, ny, fnumber);
         %DEBUG
```

```
            disp (['Finished analysis of file #',
num2str(fnumber), '.']);
            %load current_data.mat 'current_data';
            write_data(current_data, current_datafile);
        else     %summary needed
            %DEBUG
            [current_data, current_summary] = GetDNAData(I,
nx, ny, fnumber, filter_range);
            %load current_data.mat 'current_data';
            %load current_summary.mat 'current_summary';
            write_summary (current_summary, sfname);
            if summary_only ~= 1
               write_data(current_data, current_datafile);
            end
        end
   end
end % of the main for loop
num_processed = numfiles - num_error;

%======================end function AnalyseDNA()
=======================================

%==========================================================
===============================
function result = add_error_msg(filename, msg)
% adds string MSG to an errorfile FILENAME
% returns 1 if success, 0 if failure

err_FID = fopen(filename, 'at');
if err_FID == -1
   warning(['Can not open error file ' filename]);
else
   fprintf(err_FID, '%s\n', msg);
   fclose(err_FID);
```

```
end
%======================end function add_error_masg()
==================================

%========================================================
==============================
function N = getfilenumber(fname)
% returns the first number extracted from a file name
(string) or -1 if fails to extract any number
numbers = NumbersFromString( getfname(fname) ); % vector
of all numbers encoded in the name

                          % (but not in the path, even if
present)
if isempty(numbers)
   N = (-1);   % return -1 if no numbers found in the
name
else
   N = numbers(1);
end

%==================== end function getfilenumber()
===================================

%========================================================
==============================
function result = write_data(data_array, file_name)
% writes data in a data_array in a tab-delimited ascii
file.
% result is 0 if success and -1 if failure
% if file_name exists, overwrites it
result = -1;
try
   fid = fopen(file_name, 'wt');
```

```
    if fid ~= -1
       for k = 1:size(data_array, 1)
          fprintf(fid, '%g\t', data_array(k, :) );
          fprintf (fid, '\n');
       end
       test = fclose(fid);
       result = -1;
catch
    result = -1;
end

%==================== end function write_data()
=======================================

%=========================================================
===============================
function result = write_summary (s_vector, file_name)
% appends summary vector s_vector to a file_name (ASCII
tab-delimited file).
% if file_name does not exist, creates it.
% result is 0 if success and -1 if failure
%
result = -1;
try
   % debug
   fid = fopen(file_name, 'at');
   result = fprintf(fid, '%g\t', s_vector);
   result = fprintf(fid, '\n');
   result = fclose(fid);
   result = 0;
catch
   result = -1;
end
```

```
% ==================== end function write_summary()
===================================

function Data = GetObjectsData(I, Ilabel)
% GetObjectsData returns array measurements of objects in
image "I" masked by "Ilabel"
% EV 2-3-99; 2-10-99
% OData = GetObjectsData(I, Ilabel) returns an array of
morphological and intensity measurements
%    taken from a grayscale image "I". Objects are
identified on a mask image Ilabel, usually
%    created by bwlabel()
% OUTPUT:
% Each row in the output array OData represents
individual object
% columns  contain the following measurements:
%
%    1 - Index ("number" of an object);        8  -
Solidity;
%    2 - X coordinate of the center of mass; 9  - Extent;
%    3 - Y coordinate          -"-           ; 10 - Total
Intensity;
%    4 - Total Area (in pixels);              11 - Avg.
Intensity;
%    5 - Ratio of MajorAxis/MinorAxis;        12 - Median
Intensity;
%    6 - Eccentricity;                    13 - Intensity of
20% bright pixel
%    7 - EquivDiameter;                   14 - Intensity of
80% bright pixel
%
% For details on morphological parameters see information
on MatLab imfeature();
```

```
% Intensity parameters are either obvious or are
documented in comments in this file.
% Procedures in this file are documented in notebook file
"MATLAB Measuring Nuclei (1) 1-29-98.doc"

if (nargin ~= 2)
   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
   error ('function has 1 output argument (array X by
14)');
end

% finished checking arguments

% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
'MajorAxisLength',...
   'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
...
   'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
for k=1:numobjects
      OData(k, 1) = k;
      OData(k, 2) = ImStats(k).Centroid(1);
```

```
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
    OData(k, 5) = (ImStats(k).MajorAxisLength) / (ImStats(k).MinorAxisLength);
    OData(k, 6) = ImStats(k).Eccentricity ;
    OData(k, 7) = ImStats(k).EquivDiameter;
    OData(k, 8) = ImStats(k).Solidity;
    OData(k, 9) = ImStats(k).Extent;

    % now collect and assign intensity parameters from image I

    object_pixels = find( Ilabel == k);
    object_area = size(object_pixels, 1); %same as total number of pixels in the object
    object_intensities = double(I(object_pixels)); % need to convert to double to do math
    sorted_intensities = sort(object_intensities); % will need to get median, 20% and 80% pixels
    total_intensity = sum(object_intensities, 1);
    avg_intensity = total_intensity / object_area;
    median_intensity = sorted_intensities( floor( object_area/2 ) + 1 );
    pix20 = sorted_intensities( floor(object_area*0.2)+1 ) ; %brightest pixel among dimmest 20%
    pix80 = sorted_intensities( floor(object_area*0.8)+1 ) ;

    OData(k, 10) = total_intensity;
    OData(k, 11) = avg_intensity;
    OData(k, 12) = median_intensity;
    OData(k, 13) = pix20; %brightest pixel among dimmest 20%
```

```
        OData(k, 14) = pix80; %dimmest pixel among brightest
    20%
    end %for

    %==================== end function
    GetObjectsData()================================


    function Imask = MaskDNA1(I);
    % MaskDNA1 - generates binary mask for cell nuclei
    through edge detection
    % EV 1-22-99; 2-6-99; 2-10-99
    % Imask = MaskDNA1(I)
    % PARAMETERS
    %     I - intensity image (grayscale)
    % OUTPUT
    %     Imask - BW image with objects from I
    %
    % For more details see Notebook Matlab_DNA_masking1_1-22-
    99.doc
    % Uses SDC Morphology Toolbox V0.7

    if (nargin ~= 1)
       error('Wrong number of input parameters');
    end
    if (nargout ~= 1)
       error('Wrong number of output parameters: one output
    argument should be provided');
    end


    Imask = edge(I, 'canny');
    Imask = mmdil(Imask, mmsecross(1));
    Imask = mmero ( mmclohole(Imask,mmsecross(1)));
```

```
Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
Imask = medfilt2(Imask, [5 5]);

%=========end MaskDNA1 ==================================
```

Given the list of image files or montages of images as an input, this program creates an individual file for each image that contains the following quantitative measurements for all objects identified in the image:

1 - Index ("number" of an object);
2 - X coordinate of the center of mass;
3 - Y coordinate -"- ;
4 - Total Area (in pixels);
5 - Ratio of MajorAxis/MinorAxis;
6 - Eccentricity;
7 - EquivDiameter;
8 - Solidity;
9 - Extent;
10 - Total Intensity;
11 - Avg. Intensity;
12 - Median Intensity;
13 - Intensity of 20% bright pixel
14 - Intensity of 80% bright pixel

A fragment of an output for a single file, containing 9 images of cells stained for DNA and acquired with a 10x objective. A montage image that was used as a source to generate data in A is presented in Fig. 17.

The same program also summarizes measurements across many files and performs statistical analysis of the summary data. It creates a summary file with the following data:

1 - Image file number;
2 - Average object Area (in pixels);
3 - STD (standard deviation) of 2;
4 - Avg. of Ratio of MajorAxis/MinorAxis;
5 - STD of 4;
6 - Avg. Eccentricity;
7 - STD of 6;
8 - Avg. EquivDiameter;
9 - STD of 8;
10 - Avg. of Solidity;
11 - STD of 10;

12 - Avg. of Extent;	13 - STD of 11
14 - Avg. of objects Total Intensity;	15 - STD of 14
16 - Avg. of objects Avg Intensity;	16 - STD of 15
18 - Avg. of objects Median intensity;	19 - STD of 18
20 - Avg. of objects intensity of 20% bright pixel;	21 - STD of 19
22 - Avg. of objects intensity of 80% bright pixel;	23 - STD of 21

An example of summary output obtained by running AnalyseDNA against 10 montage files also is shown in Appendix B.

A seventh experiment was conducted in order to use sequence analysis algorithms to analyze features of cell images. In this experiment, HeLa cells were treated for 24 hours with several different compounds, and then fixed, and stained with a fluorescent DNA dye. One image of these cells was acquired for each of the treatments and morphometric parameters and features were measured:

Resulting measurements were arranged into a string of numbers and reduced to a pseudo- nucleic acid sequence using following rules: At any given position in the sequence a number was substituted by "t" (a code for thymidine) if its value is among highest 25% of the values at the corresponding position in the data set, "g" if it is between 50% and 25%, "c" if it is between 75% and 50%, and "a" if it belongs to lowest 25% of values. Thus one descriptor or sequence was generated per treatment as illustrated in Fig. 18.

Resulting sequences were clustered using an AlignX module commercial software package Vector NTI (http://informaxinc.com), which uses a Neighbor Joining algorithm for sequence clustering.

The resulting dendrogram is presented in Fig 18. On the dendrogram the closest "leafs" correspond to the closest pseudo-sequences. Interestingly, compounds with similar mechanisms of action cluster together on the dendrogram. Another example of the generation of pseudo-sequences and clustering is shown in Fig. 19.

In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs such as clinical trial and patient response information will be used in a similar fashion as the pre-clinical

information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions can be able to provide predictive information for this aspect of drug development.

Although the above has generally described the present invention according to specific systems. the present invention has a much broader range of applicability. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives.

Appendix A

EV Table 1.doc

Example of the output of AnalyseDNA.m program
(measurements for a single 3 by 3 montage image)

File#	Subimage	object#	X coord	Y coord.	Area	Axes ratio	Eccentricity	EquvDiam	Solidity	Extent	Intensity	Avg. Intens.	median Intens.	20% pix.	80% pix
10	1	1	12.2097	152.655	145	1.17293	0.322624	13.5875	0.923567	0.739796	4605	31.7586	34	25	37
1	1	2	16.352	416.032	125	1.60594	0.782471	12.6157	0.905797	0.78125	4606	36.848	38	30	45
1	1	3	20.1073	73.8079	177	1.09845	0.413785	15.0121	0.917098	0.691406	4769	26.9435	29	22	31
1	1	4	21.4186	402.744	43	1.36215	0.679004	7.39928	0.914894	0.767857	3690	85.814	87	67	105
1	1	5	27.0938	104	96	1.30887	0.645194	11.0558	0.888889	0.671329	4502	46.8958	49	38	56
1	1	6	30.3252	259.534	206	2.33106	0.903309	16.1953	0.927928	0.715278	6380	30.9709	33	24	37
1	1	7	33.6629	187.573	89	1.34984	0.671696	10.6451	0.927083	0.741667	4225	47.4719	50	39	56
1	1	8	35.0411	16.9726	146	1.25176	0.601495	13.6343	0.929936	0.748718	5415	37.089	40	29	44
1	1	9	37.766	366.021	47	1.84062	0.839542	7.73578	0.87037	0.652778	6667	141.851	142	113	171
1	1	10	49.1078	370.004	232	1.90491	0.851127	17.187	0.852941	0.70303	9832	42.3793	45	33	51
1	1	11	56.0749	126.534	221	1.95708	0.859595	16.7746	0.924686	0.686335	7040	31.8552	33	25	37
1	1	12	52.7755	44.9932	147	1.33627	0.661301	13.6809	0.907407	0.706731	4765	32.415	34	24	39
1	1	13	53.8444	364.854	171	2.27225	0.897953	14.7555	0.872449	0.706612	9378	54.8421	56	43	68
1	1	14	58.4029	282.272	206	1.92782	0.854944	16.1953	0.923767	0.64375	7137	34.6456	37	28	43
1	1	15	57.0648	227.176	108	1.73885	0.818088	11.7265	0.915254	0.701299	4644	43	45	33	53
1	1	16	68.3714	333.181	315	1.11194	0.437266	20.0267	0.75	0.526756	15151	48.0984	50	36	62
1	1	17	65.1409	402.414	220	1.70147	0.80906	16.7366	0.970502	0.647059	9809	44.5864	46	35	54
1	1	18	71.8449	443.13	185	1.77678	0.826583	15.3476	0.91133	0.649123	6124	33.1027	35	25	39
1	1	19	73.626	184.854	123	1.71588	0.812672	12.5143	0.911111	0.723529	4841	39.3577	41	30	47
1	1	20	77.4869	132.513	306	1.6379	0.791988	19.7386	0.822581	0.632231	14559	47.5784	50	38	57
1	1	21	78.7377	208.27	122	1.3357	0.662941	12.4634	0.910448	0.739394	4483	36.7459	40	29	43
1	1	22	81.4786	53.5012	117	1.68713	0.794614	12.2053	0.886364	0.642857	4686	40.0513	43	32	47
1	1	23	89.7292	281.534	373	2.17388	0.887916	21.7926	0.843091	0.531339	16109	43.1877	46	34	52
1	1	24	84.1765	341.976	85	1.20799	0.560991	10.4031	0.876289	0.708333	4509	53.0471	57	43	63
1	1	25	88.1608	176.231	143	1.43513	0.717545	13.4935	0.940789	0.794444	4878	34.1119	35	27	41
1	1	26	91.4529	376.924	170	1.36853	0.682689	14.7123	0.909721	0.833333	4933	29.0176	30	23	35
1	1	27	97.7604	317.795	288	1.97353	0.862119	19.1492	0.9	0.606316	10663	37.0243	39	29	45
1	1	28	96.5841	230.363	113	1.09835	0.413609	11.9948	0.896825	0.668639	4540	40.177	43	33	48
1	1	29	96.9492	248.602	118	1.2774	0.622219	12.2573	0.921875	0.766234	4873	41.2966	43	32	51
1	1	30	101.033	93.3279	122	1.48415	0.738927	12.4634	0.945736	0.813333	4663	38.2213	40	31	47
1	1	31	103.47	155.507	134	1.5708	0.753431	13.0619	0.937008	0.687179	4358	32.5224	34	27	38
1	1	32	105.356	57.1271	118	1.90339	0.850869	12.2573	0.907692	0.694118	4695	39.7881	42	30	49
1	1	33	121.23	285.08	326	1.70379	0.809514	20.3734	0.900552	0.626923	15864	48.6626	50	39	60
1	1	34	125.532	170.645	141	1.53045	0.757011	13.3988	0.927632	0.734375	6429	45.5957	49	37	53
1	1	35	128.98	60.3355	152	1.75689	0.822708	13.9116	0.921212	0.747417	6875	45.2303	47	36	54
1	1	36	137.083	128.083	266	1.845	0.840375	18.4033	0.796407	0.538462	9810	36.8797	38	26	45
1	1	37	130.902	411.5	164	1.19276	0.54507	14.4503	0.937143	0.788462	7337	44.7378	47	35	53
1	1	38	137.439	352.545	187	1.39705	0.698312	15.4304	0.935	0.799145	5227	27.9519	27	22	34
1	1	39	129.615	16.6154	13	1.15921	0.50579	4.06843	0.928571	0.8125	94	7.23077	8	3	11
1	1	40	136.574	209.059	101	1.17013	0.450545	11.3401	0.87069	0.647436	7293	72.2079	74	58	88
1	1	41	134.455	33.0909	33	1.21149	0.564506	6.48204	0.916667	0.785714	1949	59.0606	60	44	75
1	1	42	140.833	102.008	121	1.47787	0.736206	12.4122	0.916667	0.733333	4808	39.7355	40	32	47
1	1	43	144.864	59.8199	272	1.75519	0.821825	18.6097	0.924708	0.725333	10814	39.7574	41	32	48
1	1	44	147.093	438.435	161	1.3071	0.64397	14.3175	0.925287	0.715556	9000	55.9006	58	44	68
1	1	45	151.46	256.924	224	1.08008	0.377872	16.888	0.937238	0.777778	9654	43.0982	45	33	53
1	1	46	155.688	178.546	141	1.39135	0.695293	13.3988	0.921569	0.801136	7729	54.8156	57	44	65
1	1	47	160.875	342.354	48	1.71551	0.812532	7.81764	0.851143	0.592593	6609	137.688	139	103	168
1	1	48	168.828	11.7677	198	1.42126	0.710595	15.8777	0.933962	0.733333	9931	50.1566	50	39	62
1	1	49	169.613	136.267	217	1.74719	0.820008	16.6221	0.825095	0.5425	7980	36.7742	38	28	45
1	1	50	176.014	356.414	222	1.44388	0.720419	16.8125	0.790036	0.621849	9626	43.3604	46	33	54
1	1	51	175.788	192.983	118	1.13784	0.47708	12.2573	0.900763	0.698225	4671	39.5847	42	32	47
1	1	52	177.181	210.874	127	1.14539	0.487606	12.7162	0.92029	0.755952	4862	38.2835	40	30	45
1	1	53	178.367	410.524	147	1.24127	0.592425	13.6809	0.924528	0.75	9096	61.8776	67	50	72
1	1	54	182.4	392.476	170	1.57768	0.773587	14.7123	0.939227	0.714286	9335	54.9118	58	44	66
1	1	55	189.388	262.719	196	1.81704	0.834936	15.7973	0.915888	0.742424	5030	25.6633	27	20	31
1	1	56	200.742	93.7418	213	1.36983	0.683428	16.4682	0.934211	0.747368	9665	45.3756	48	38	53
1	1	57	199.198	136.725	91	1.04663	0.295161	10.7641	0.90099	0.752066	4188	46.022	47	36	56
1	1	58	209.61	185.871	264	1.96083	0.860187	18.334	0.916667	0.6	9847	37.2977	39	29	46
1	1	59	208.752	70.0435	230	1.69129	0.806478	17.1127	0.946502	0.804196	9766	42.4609	44	34	53
1	1	60	212.584	368.695	197	1.13679	0.475587	15.8376	0.929245	0.724265	7014	35.6041	36	27	44
1	1	61	220.856	30.6237	194	2.47642	0.927588	15.7165	0.92823	0.776	4635	23.8918	25	18	29
1	1	62	216.568	234.29	183	1.34183	0.666785	15.2644	0.943299	0.828054	5078	27.7486	28	21	34
1	1	63	216.292	293.953	171	1.64627	0.79437	14.7555	0.919355	0.692308	5053	29.5497	30	23	35
1	1	64	217.333	330.721	172	1.72537	0.81491	14.7986	0.924731	0.671875	5040	29.3023	31	22	36
1	1	65	217.388	427.1	201	1.79372	0.834448	15.9975	0.926267	0.738971	6094	30.3184	32	25	36
1	1	66	222.926	157.769	121	1.3261	0.656763	12.4122	0.916667	0.733333	4352	35.9669	38	29	42
1	1	67	249.071	408.968	435	1.20117	0.549955	23.5342	0.74359	0.402778	14810	34.046	37	26	42
1	1	68	238.844	265.186	167	1.48408	0.738897	14.5819	0.938202	0.755656	5158	30.8862	32	25	37

69	243.509	88.7857	224	1.87991	0.846782	16.888	0.899598	0.636364	8827	39.4063	42	30	49
70	248.831	322.144	160	1.79581	0.830819	14.273	0.91954	0.76555	5025	31.4063	33	25	38
71	249.234	413.026	77	1.15766	0.498851	9.90149	0.875	0.7	4352	56.5135	59	16	63
72	256.945	43.816	163	1.58857	0.777002	14.4062	0.91573	0.678167	4720	28.9571	30	23	34
73	257.061	398.848	66	1.03149	0.245208	9.167	0.916667	0.814815	4440	67.2727	71	56	80
74	263.602	375.59	251	1.95991	0.860039	17.8769	0.888926	0.597619	10500	41.8327	43	32	50
75	264.292	233.801	161	1.65084	0.795652	14.3175	0.914773	0.688034	5136	31.9006	33	25	38
76	264.937	209.807	111	1.31512	0.649472	11.8882	0.917355	0.720779	4858	43.7658	45	36	52
77	266.137	340.328	131	1.50185	0.774829	12.9149	0.909722	0.682292	9633	73.5344	77	56	89
78	276.221	171.24	204	2.05613	0.873763	16.1145	0.918919	0.596491	7051	34.5637	35	28	41
79	277.059	285.098	287	1.27833	0.622935	19.114	0.87234	0.650794	10520	36.6551	38	29	45
80	278.321	97.32	150	1.10321	0.422329	13.8134	0.920245	0.765306	9202	61.3467	63	42	78
81	276.612	391.118	85	1.67318	0.801747	10.4031	0.923913	0.817308	4387	51.6118	55	43	62
82	285.905	154.719	231	1.56301	0.74855	17.1498	0.931452	0.675439	8580	37.1429	39	31	44
83	285.326	203.608	224	1.75638	0.822052	16.7716	0.863281	0.701587	10251	46.3846	49	34	57
84	286.739	355.022	46	1.74017	0.818394	7.65306	0.867925	0.730159	6986	151.87	159	120	185
85	291.4	319.71	145	1.33364	0.648467	13.5875	0.917722	0.755208	6940	36.069	35	27	42
86	293.651	442.734	192	2.01531	0.868208	15.6353	0.911176	0.793388	5912	31.1042	32	23	39
87	293.81	389.276	58	1.25774	0.60651	8.59368	0.920635	0.725	3964	68.3418	70	55	82
88	299.182	285.182	159	1.39283	0.696112	14.2283	0.929825	0.757143	5103	32.0943	33	26	39
89	300.14	356.347	150	1.31538	0.649613	13.8198	0.925926	0.78125	5369	35.7933	37	30	41
90	311.5	260.38	382	1.57517	0.772632	22.054	0.843267	0.598746	14117	42.1911	44	32	53
91	308.77	132.894	161	1.15063	0.49465	14.3175	0.936047	0.766667	4966	30.8447	33	24	38
92	317.161	21.8111	180	1.62866	0.789304	15.1388	0.932642	0.728745	4915	27.3056	28	22	32
93	308.683	204.548	126	1.6436	0.793615	12.464	0.9	0.7	4950	39.2857	40	31	48
94	317.72	43.06	150	2.18043	0.88863	13.8198	0.882353	0.595238	4958	33.0533	35	25	41
95	315.779	222.448	145	1.2166	0.569542	13.5875	0.917722	0.74359	5048	34.8138	36	28	42
96	314.612	396.048	147	1.78365	0.828055	13.6809	0.936306	0.773684	9195	62.551	65	47	78
97	319.331	79.6031	283	1.31849	0.608294	17.5497	0.915525	0.759335	8192	33.7119	36	27	39
98	321.995	171.85	207	1.13294	0.52263	16.2345	0.918251	0.761029	4993	24.1208	25	19	29
99	327.232	313.626	89	1.27961	0.623918	11.2272	0.933962	0.761538	4632	48.7879	50	35	57
100	328.895	465.333	153	1.61993	0.786719	13.9573	0.9	0.6375	4428	28.9412	30	22	36
101	339.875	30.4024	328	2.08757	0.877801	20.4358	0.896175	0.594203	10208	31.122	33	24	38
102	336.286	348.101	168	1.36659	0.681577	14.6255	0.928177	0.746667	4848	28.8571	29	23	35
103	335.277	126.088	137	1.47067	0.733247	13.2073	0.901316	0.713542	6932	50.5985	53	41	59
104	336.608	376.692	130	1.44662	0.722601	12.8655	0.902778	0.666667	4713	36.2538	38	29	43
105	340.273	422.859	128	1.82797	0.837097	12.7662	0.920863	0.68841	4609	36.0078	38	29	43
106	341.426	392.101	149	1.21343	0.566431	13.7736	0.925466	0.764103	4920	33.0201	35	27	38
107	353.93	211.249	201	1.50656	0.767942	15.9975	0.922018	0.773077	8487	42.2239	43	34	51
108	352.982	107.863	170	1.49726	0.746243	14.7123	0.909091	0.688259	4955	29.1471	30	23	36
109	360.316	246.848	152	1.86075	0.843317	13.9116	0.894218	0.596078	5109	33.6118	35	25	41
110	357.882	436.327	110	1.20428	0.557215	11.8345	0.901639	0.714286	4290	39	41	30	48
111	361.836	318.483	116	1.51165	0.749922	12.153	0.885496	0.644444	4620	39.8276	42	32	48
112	361.357	65	140	1.27314	0.618912	13.3512	0.933333	0.777778	4854	34.6714	35	27	43
113	363.058	282.398	103	1.26774	0.614641	11.4518	0.927928	0.792308	4724	45.8641	47	36	56
114	377.556	52.6893	383	2.24284	0.895101	22.0828	0.851111	0.526099	12083	31.5483	30	23	44
115	369.901	366.09	111	1.32541	0.656322	11.8882	0.909836	0.776224	4428	39.8919	41	31	48
116	372.355	389.358	183	1.08317	0.392338	15.2644	0.953125	0.871429	4673	25.5355	26	19	32
117	375.494	102.31	168	1.77626	0.82647	14.6255	0.913043	0.658824	6590	39.2262	41	32	48
118	377.238	164.669	172	1.55567	0.766024	14.7986	0.910053	0.67451	5008	29.1163	30	24	35
119	378.105	487.237	190	1.36387	0.668045	15.5536	0.913462	0.698529	6078	31.9895	32	23	41
120	386.803	125.403	142	1.82726	0.838959	13.4462	0.940397	0.788845	8149	57.3873	61	48	69
121	387.01	222.932	206	1.31589	0.751317	16.1953	0.923767	0.792308	10014	48.6117	50	37	60
122	384.354	305.719	96	1.74508	0.819527	11.0558	0.923077	0.666667	4564	47.5417	48	36	59
123	397.856	400.719	313	1.71338	0.812011	19.9631	0.800512	0.610136	14036	44.8435	46	36	56
124	389.741	281.944	108	1.86676	0.844417	11.7265	0.892562	0.593407	4668	43.2222	44	33	53
125	393.218	318.762	105	1.49331	0.742673	11.5624	0.913043	0.673077	4613	43.9333	46	36	52
126	395.708	20.375	120	1.06486	0.34362	12.3608	0.916031	0.765231	4538	37.8167	39	32	45
127	402.593	198.495	194	1.83729	0.838903	15.7165	0.932692	0.769841	9702	50.0103	52	39	61
128	403.562	362.046	130	2.18482	0.889105	12.8655	0.902778	0.401852	4566	35.1231	37	28	43
129	402.298	35.0744	121	1.34169	0.666895	12.4122	0.909774	0.720238	4622	38.1983	41	31	45
130	407.174	349.358	109	1.26316	0.610958	11.7806	0.908332	0.698718	4433	40.6697	42	31	49
131	410.441	432.387	142	2.10237	0.879632	13.4462	0.898734	0.71	4284	30.169	32	23	37
132	408.948	170.017	174	1.13507	0.717235	14.8843	0.910995	0.74359	4845	27.8448	29	21	35
133	415.673	271.104	147	1.36813	0.682628	13.6809	0.93038	0.765625	4976	33.8503	36	28	39
134	419.881	320.856	118	1.70261	0.809346	12.2573	0.887218	0.605128	4596	38.9492	41	31	48
135	420.797	224.645	172	1.24283	0.593793	14.7986	0.92973	0.764444	5120	29.7674	32	24	35
136	428.437	38.3016	126	1.24139	0.593057	12.666	0.928471	0.75	4585	36.3889	39	30	43
137	434.977	106.932	222	1.74232	0.818891	16.8125	0.925	0.720779	7043	31.7252	33	25	38
138	432.808	421.282	131	1.70121	0.808993	12.9149	0.922535	0.770588	4251	32.4504	33	24	40
139	431.155	477.519	129	1.14151	0.48225	12.8159	0.914894	0.767857	4387	34.0078	35	27	41
140	438.675	12.7222	126	1.21999	0.424707	12.666	0.933333	0.75	4445	35.2778	38	26	42
141	441.29	464.766	124	1.33653	0.662198	12.5651	0.918519	0.688889	4658	37.5645	40	30	45
142	445.287	152.375	134	1.30301	0.690787	13.159	0.912752	0.708333	5017	36.8897	39	28	44
143	452.32	495.598	306	1.66314	0.799015	19.7366	0.918919	0.796875	17726	57.9281	61	47	68
144	451.122	370.69	288	1.77678	0.826582	19.1492	0.825215	0.659039	11368	39.5417	42	30	49

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1	1	145	462.718	316.942	291	2.5997	0.923028	19.2487	0.716719	0.534926	9565	37.0694	32	23	43
1	1	146	455.84	345.437	319	1.51711	0.752012	12.3092	0.915365	0.793333	4473	37.5892	39	28	46
1	1	147	459.357	398.325	158	1.73315	0.816025	14.0028	0.922156	0.712863	8038	52.1948	55	42	62
1	1	148	459.428	30.199	201	1.48473	0.739168	15.9975	0.934888	0.755638	4615	22.9602	24	18	27
1	1	149	462.472	163.039	127	1.2763	0.621371	12.7162	0.917761	0.824675	4849	38.1011	40	30	48
1	1	150	465.094	238.432	405	1.32102	0.653423	22.7082	0.89404	0.771429	9811	24.2247	25	19	29
1	1	151	459	185	23	1.22522	0.577956	5.41152	0.851852	0.657143	2687	116.826	118	86	135
1	1	152	469.289	73.6401	228	1.65352	0.796399	17.0382	0.919355	0.890909	8169	35.8289	37	28	44
1	1	153	468.291	365.573	117	1.37288	0.685157	12.7053	0.936	0.835714	4553	38.9145	40	32	45
1	1	154	465.261	188.174	23	1.17386	0.523119	5.41152	0.92	0.766667	2572	111.826	115	97	134
1	1	155	477.09	285.407	199	1.33007	0.666436	15.9177	0.947619	0.737037	7638	38.3819	40	31	46
1	1	156	480.109	128.384	138	1.47131	0.733523	13.2555	0.926174	0.704082	4514	32.7101	34	27	39
1	1	157	485.215	212.949	162	1.99846	0.865802	14.4062	0.91573	0.679167	6791	41.6626	43	33	51
1	1	158	493.535	460.984	129	1.46523	0.730898	12.8159	0.921429	0.716667	4590	35.5814	37	29	44
1	1	159	490.198	257.492	197	1.18457	0.536017	15.8376	0.933649	0.769531	6333	32.1371	34	25	38
1	1	160	498.107	428.713	122	1.39078	0.694967	12.4634	0.910448	0.739398	4778	39.1639	40	31	48
1	1	161	501.794	480.579	107	1.30083	0.639559	11.672	0.938596	0.743056	4301	40.1963	42	32	47
1	2	1	16.0133	189.207	150	1.82513	0.83651	13.8198	0.9375	0.789474	5013	33.42	35	26	40
1	2	2	13.3492	92.619	126	1.24511	0.595788	12.666	0.919708	0.75	4636	36.7937	39	31	44
1	2	3	12.7436	254.949	78	1.23696	0.588585	9.96557	0.906977	0.797879	4177	53.5513	54	42	65
1	2	4	21.4605	27.5132	152	2.05915	0.874161	13.9116	0.915663	0.59375	5398	35.5132	38	29	42
1	2	5	24.5089	412.717	180	1.41069	0.705337	15.1388	0.923077	0.714286	7613	42.2944	45	33	52
1	2	6	30.9013	150.013	151	1.5312	0.757285	13.9658	0.937888	0.807487	4834	32.0132	34	25	39
1	2	7	35.9214	117.641	153	1.10365	0.42309	13.9573	0.916168	0.728571	8847	57.8235	60	46	70
1	2	8	32.4386	483.965	57	1.17959	0.530388	8.51908	0.901762	0.791667	7839	137.526	142	104	175
1	2	9	41.1142	68.0551	254	2.15977	0.986352	17.9034	0.927007	0.575964	9532	33.5906	36	27	39
1	2	10	46.0943	205.164	159	1.43069	0.715156	14.2283	0.929825	0.779412	5043	31.717	32	25	38
1	2	11	47.0543	98.2403	129	1.6944	0.807273	12.8159	0.921429	0.661538	4858	37.6589	39	28	46
1	2	12	48.3996	164.971	138	1.40133	0.700548	13.2555	0.924174	0.784091	4896	35.4783	38	28	42
1	2	13	54.1071	128.629	140	1.31888	0.652002	13.3512	0.921053	0.777778	3556	53.9714	56	47	63
1	2	14	61.2347	252.877	375	1.46523	0.730898	21.851	0.860056	0.652174	18748	48.6613	52	38	60
1	2	15	58.7059	420.598	102	1.16735	0.515812	11.3961	0.907655	0.713287	8303	61.402	84	69	96
1	2	16	62.0217	461.434	296	1.48931	0.723873	18.0826	0.925566	0.654462	10326	36.1049	38	28	45
1	2	17	68.6789	183.686	194	1.09744	0.949849	15.7165	0.92823	0.769841	7275	37.5	39	29	47
1	2	18	72.3873	25.8873	204	1.65928	0.797891	16.3165	0.894737	0.647619	4906	24.049	26	18	29
1	2	19	83.1654	62.6808	260	1.92679	0.854775	18.1946	0.9319	0.656566	10031	38.5408	40	31	47
1	2	20	96.7188	481.695	256	2.0471	0.872567	19.0541	0.885013	0.593593	9587	37.4492	37	29	43
1	2	21	94.0154	108.485	130	1.50401	0.746944	12.8655	0.915493	0.738636	4616	35.5077	36	26	44
1	2	22	101.038	140.038	207	1.85907	0.797932	16.2345	0.924107	0.758242	9618	46.4638	48	37	56
1	2	23	106.469	179.984	254	2.25068	0.995873	17.9934	0.92029	0.552178	9484	37.3386	40	29	46
1	2	24	118.615	415.286	455	2.21916	0.995724	24.0692	0.803887	0.530303	19707	43.3121	45	31	56
1	2	25	109.325	467.783	157	1.33362	0.661618	14.1386	0.934524	0.769608	7940	50.5732	53	38	62
1	2	26	112.274	30.2115	156	1.68158	0.803963	14.0935	0.912281	0.65	4684	30.0256	31	24	37
1	2	27	114.738	94.3308	130	2.20139	0.89087	12.8655	0.866667	0.546218	4593	35.3308	36	27	45
1	2	28	113.664	187.473	131	1.89702	0.869777	12.9149	0.879195	0.582222	4551	34.7405	35	27	42
1	2	29	119.497	260.882	195	2.28309	0.858974	15.757	0.928571	0.633117	9042	46.3692	50	36	55
1	2	30	120.542	161.685	203	1.63181	0.790275	16.0769	0.931193	0.732281	9555	47.069	48	37	58
1	2	31	125.017	493.152	302	2.03965	0.871565	19.6091	0.92638	0.770408	14405	54.3212	57	43	66
1	2	32	128.12	375.544	125	1.54837	0.7634	12.6157	0.919118	0.688813	4694	37.552	39	29	46
1	2	33	134.357	246.786	182	1.30577	0.643043	15.2227	0.943005	0.764706	9424	51.7802	54	42	62
1	2	34	134.824	435.275	182	1.39127	0.69525	15.2721	0.933333	0.713725	9568	52.5714	55	42	63
1	2	35	137.463	477.361	205	1.67189	0.802393	16.3559	0.911111	0.719298	10237	49.4488	50	38	61
1	2	36	140.229	112.255	275	2.43852	0.912047	19.7121	0.813609	0.458333	9908	36.0291	38	27	45
1	2	37	141.759	62.396	303	2.71691	0.932909	19.6416	0.733656	0.510101	9815	32.3927	35	25	39
1	2	38	142.944	349.208	125	1.15103	0.495153	12.6157	0.925926	0.801282	4627	37.016	38	30	44
1	2	39	155.234	38.7654	410	2.62596	0.924652	22.8179	0.742754	0.615822	14441	35.222	36	25	47
1	2	40	145.827	141.547	150	1.38574	0.692274	13.8198	0.914634	0.721154	5019	33.44	35	26	40
1	2	41	171.118	418.674	144	2.13773	0.883842	13.5406	0.9	0.571429	4475	31.0764	32	24	38
1	2	42	169.885	483.943	218	2.03707	0.871215	16.6603	0.904564	0.619318	4735	21.7202	23	17	26
1	2	43	173.468	380.368	171	1.76541	0.824104	14.7555	0.909574	0.7125	4792	28.0234	30	21	34
1	2	44	173.139	451.197	122	1.71693	0.812477	12.4634	0.897059	0.717647	4270	35	34	27	44
1	2	45	177.184	127.971	343	2.17611	0.88816	20.8179	0.955649	0.711503	17821	51.5563	55	42	62
1	2	46	178.925	44.5574	122	1.47137	0.735547	12.4634	0.917293	0.739336	4501	36.9934	39	30	44
1	2	47	194.558	101.051	165	1.53097	0.757203	14.4913	0.921788	0.746606	5051	30.6121	32	25	36
1	2	48	187.282	274.494	174	2.03959	0.871557	14.8843	0.910995	0.690476	4740	27.3563	29	21	33
1	2	49	191.89	384.491	163	1.86367	0.84389	14.4062	0.926136	0.679167	4539	27.8466	29	22	33
1	2	50	201.221	456.572	145	1.33621	0.663261	13.5875	0.911558	0.690476	4481	32.2828	33	24	40
1	2	51	201.162	356.394	111	1.70636	0.810281	11.8882	0.880352	0.69375	4314	38.8449	41	31	45
1	2	52	204.038	41.5031	163	2.01967	0.868819	14.4062	0.910615	0.740505	4510	27.6697	30	23	33
1	2	53	206.779	234.517	145	1.69903	0.808446	13.5875	0.90625	0.710784	4465	32.1724	32	25	39
1	2	54	209.482	328.447	114	1.21621	0.569185	12.0478	0.926429	0.730769	4550	39.9123	41	31	49
1	2	55	210.386	102.954	158	1.97132	0.861784	14.1835	0.934911	0.619808	4640	29.3671	31	23	35
1	2	56	218.981	432.981	157	2.05395	0.873476	14.1386	0.902299	0.633628	4489	28.5924	30	23	34
1	2	57	221.277	471.106	142	1.48441	0.712149	13.3988	0.933775	0.801136	4526	32.0393	33	25	39
1	2	58	223.709	401.449	134	1.06494	0.353312	13.0619	0.911565	0.726264	4524	33.7612	35	26	42
1	2	59	222.761	129.858	113	1.23854	0.590005	11.9318	0.904	0.733766	5226	46.2478	48	36	55

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1	2	60	223.098	314.61	123	1.19435	0.546784	12.5143	0.924812	0.732143	4533	36.0537	38	29	44
1	2	61	231.901	273.099	171	1.87159	0.845292	14.7555	0.921324	0.7125	5607	33.2573	35	26	40
1	2	62	230.337	380.573	178	1.64435	0.793828	15.0565	0.927093	0.698039	4786	26.8876	28	21	34
1	2	63	233.936	63.4295	298	1.96264	0.860461	19.4788	0.949003	0.820833	9681	32.4866	34	26	39
1	2	64	243.815	495.715	480	1.27812	0.623387	24.7215	0.933852	0.689655	23348	48.6333	52	40	58
1	2	65	239.465	27.8994	159	1.59375	0.774656	14.2283	0.908571	0.668067	6050	38.0503	39	29	47
1	2	66	238.785	118.85	107	1.3017	0.810175	11.672	0.90678	0.748252	5012	47.1215	48	39	56
1	2	67	248.411	107.352	219	1.76031	0.822971	16.6985	0.9327	0.608333	10198	47.9178	50	38	59
1	2	68	248.387	210.387	163	1.1782	0.528789	14.4062	0.935782	0.77619	4802	29.4601	31	22	35
1	2	69	251.401	459.551	147	1.36265	0.679415	13.6809	0.924528	0.765625	5259	35.7755	37	28	44
1	2	70	257.144	48.2734	139	1.28227	0.625943	13.3034	0.914474	0.772222	6892	49.5827	51	39	60
1	2	71	265.238	469.394	140	1.46715	0.731733	14.273	0.924455	0.784314	5318	33.2375	35	28	38
1	2	72	270.909	422.169	516	3.69314	0.962883	25.8318	0.786585	0.565789	24246	46.9884	48	31	60
1	2	73	273.05	102.567	201	2.48028	0.915121	15.9975	0.922018	0.844231	5194	25.8408	27	19	32
1	2	74	275.753	332.912	170	1.84216	0.839835	14.7123	0.918919	0.708333	4889	28.7588	30	21	37
1	2	75	282.78	24.8535	157	1.45739	0.727454	14.1386	0.94012	0.769608	8907	56.7325	59	44	68
1	2	76	304.73	201.073	178	1.19701	0.549523	15.0545	0.92228	0.747093	9829	55.2191	56	41	68
1	2	77	305.481	484.962	185	2.0845	0.877415	15.3876	0.91133	0.629252	4598	24.8432	26	19	30
1	2	78	307.247	40.9177	158	1.24996	0.599963	14.1835	0.934911	0.759815	5031	31.8418	34	26	37
1	2	79	308.276	329.299	127	1.83279	0.838036	12.7162	0.900709	0.661458	4575	36.0236	37	27	44
1	2	80	310.67	141.898	176	1.86898	0.84482	14.9696	0.931217	0.651852	4921	27.9602	29	21	34
1	2	81	315.681	247.266	229	1.52517	0.755038	17.0755	0.923387	0.724901	10311	45.0262	47	34	55
1	2	82	314.476	302.312	170	1.42819	0.713958	14.7123	0.923913	0.714286	4865	28.6176	29	23	35
1	2	83	320.461	216.509	228	1.37765	0.687826	17.0382	0.938272	0.76	10187	44.6798	46	37	53
1	2	84	320.624	423.867	224	3.36105	0.954714	16.9633	0.896825	0.431615	4603	20.3673	21	15	25
1	2	85	319.458	376.38	216	2.73428	0.930722	16.5837	0.892562	0.56	4741	21.9491	22	17	27
1	2	86	323.423	62.4261	182	1.43137	0.715481	15.2227	0.923856	0.722222	9774	53.7033	57	43	63
1	2	87	340.016	384.683	246	2.34015	0.904099	17.6979	0.81457	0.630769	9905	40.2642	43	32	49
1	2	88	344.4	270.331	285	1.43643	0.717877	19.0492	0.931373	0.714286	10324	36.2246	37	29	44
1	2	89	340.84	65.4787	94	1.19007	0.542146	10.94	0.903846	0.712121	4464	47.4894	51	37	57
1	2	90	341.06	478.91	134	2.01084	0.867577	13.0619	0.937063	0.783626	4299	32.0821	33	25	39
1	2	91	345.369	334.047	149	1.10502	0.425497	13.7736	0.925466	0.760204	4161	31.953	33	24	39
1	2	92	357.892	371.092	249	1.84813	0.840967	17.8055	0.932584	0.691667	6568	26.3775	27	21	33
1	2	93	354.205	391.672	122	1.46764	0.731944	12.4634	0.911293	0.67033	9313	76.3361	82	63	89
1	2	94	353.903	77.2419	124	1.57831	0.773669	12.5651	0.892086	0.688889	4741	38.2339	40	30	46
1	2	95	355.409	136.509	110	1.44194	0.720445	11.8343	0.916667	0.785714	4501	40.9182	42	32	49
1	2	96	361.412	208.768	194	1.20688	0.559863	15.7165	0.932692	0.760784	7985	41.1598	43	32	50
1	2	97	363.881	417.549	113	2.73659	0.930843	11.9948	0.819624	0.523148	2950	26.1062	26	16	36
1	2	98	370.626	159.489	131	1.56713	0.765949	12.9149	0.919275	0.81875	4759	36.3282	37	29	43
1	2	99	373.978	497.422	225	1.84477	0.840332	16.9257	0.918367	0.649843	9414	41.84	43	33	52
1	2	100	335.192	63.568	125	1.35276	0.673144	12.6157	0.932836	0.739645	4708	37.664	39	30	46
1	2	101	372	428.5	6	1.5	0.745356	2.76395	1	1	54	9	9	8	11
1	2	102	375.31	461.469	113	2.32052	0.902381	11.9948	0.862595	0.627778	2648	23.4336	24	17	30
1	2	103	372	441	1	1	0	1.12838	1	1	9	9	9	9	9
1	2	104	380.145	395.421	145	1.61066	0.783919	13.5875	0.917722	0.697115	4480	30.8966	32	24	38
1	2	105	384.421	107.916	202	1.72136	0.813944	16.0373	0.874459	0.590643	8500	42.0792	42	29	54
1	2	106	385.749	314.015	195	1.58739	0.776623	15.757	0.924171	0.684211	9749	49.9949	52	38	63
1	2	107	394.915	291.683	82	1.56076	0.767761	10.2179	0.87234	0.630769	7836	95.561	100	77	111
1	2	108	407.296	186.112	179	1.93382	0.855918	15.0967	0.92268	0.639286	4942	27.6089	28	20	36
1	2	109	405.333	167.802	162	1.96376	0.860631	14.3619	0.9	0.608023	4778	29.4938	31	22	36
1	2	110	405.662	54.9615	130	1.23602	0.587746	12.8655	0.915493	0.714286	4523	34.7923	36	27	42
1	2	111	409	479.357	129	1.86558	0.844201	12.8159	0.895633	0.614286	4429	34.3333	37	26	42
1	2	112	418.278	100.644	180	1.28967	0.631482	15.1386	0.891089	0.705882	7824	43.4667	47	33	53
1	2	113	426.611	225.589	270	1.81845	0.835218	18.5412	0.851089	0.613636	9758	36.1407	38	26	44
1	2	114	423.62	401.364	129	1.41093	0.705455	12.8159	0.902038	0.716667	4596	35.6279	37	27	43
1	2	115	425.799	183.281	139	1.85684	0.842594	13.3034	0.908437	0.820536	4569	32.8705	35	25	40
1	2	116	429.85	439.218	206	1.51878	0.752647	16.1953	0.938364	0.774436	7146	34.6893	35	26	43
1	2	117	425.908	491.557	131	1.44763	0.723062	12.9149	0.916084	0.744318	6530	49.8473	53	37	60
1	2	118	434.563	58.8768	138	1.25228	0.601938	13.2555	0.926174	0.704082	4540	32.8986	35	26	39
1	2	119	439.22	364.115	218	2.32616	0.90288	16.6603	0.875507	0.605556	9123	41.8486	43	33	52
1	2	120	433.784	385.392	51	1.25129	0.601101	8.05824	0.944444	0.796875	3773	73.9804	72	60	89
1	2	121	436.309	477.414	152	1.08613	0.390878	13.9116	0.91018	0.72381	4441	29.2368	30	23	35
1	2	122	446.156	156.905	147	1.93636	0.856326	13.6809	0.93038	0.773684	4785	32.551	34	25	39
1	2	123	448.056	77.784	162	1.62655	0.788684	14.3619	0.9	0.710526	4549	28.0802	30	22	34
1	2	124	446.81	389.738	42	1.13949	0.479424	7.31273	0.893617	0.75	3482	82.9048	87	67	104
1	2	125	452.475	39.766	141	1.54502	0.769232	13.3988	0.933775	0.734375	8316	58.9787	61	47	71
1	2	126	457.992	123.558	129	1.35097	0.672379	12.8159	0.921429	0.732955	4753	36.845	38	27	46
1	2	127	457.668	492.056	124	1.37051	0.683818	12.5651	0.918519	0.751515	4109	33.1371	34	26	41
1	2	128	463.253	30.4967	151	1.82568	0.788427	13.8658	0.937888	0.725962	7643	50.6159	53	40	60
1	2	129	466.371	220.547	232	1.63718	0.791781	17.187	0.924303	0.682353	9151	39.444	41	31	48
1	2	130	470.772	77.88	250	3.50176	0.958431	17.8412	0.859107	0.434783	9444	37.776	38	29	47
1	2	131	472.497	344.745	145	1.74711	0.819994	13.5875	0.889571	0.65378	4916	33.9034	35	27	41
1	2	132	474.284	440.578	109	1.72316	0.816383	11.7806	0.923729	0.707752	4014	36.8257	39	29	43
1	2	133	475.31	456.124	123	1.32543	0.656329	11.9948	0.918699	0.733766	3961	35.0531	37	28	42
1	2	134	474.222	407.025	81	1.22035	0.573208	10.1554	0.9	0.736364	2413	29.7901	32	24	36
1	2	135	491.61	392.063	205	2.29749	0.900306	16.1555	0.931818	0.774515	5209	25.4098	26	19	32

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1	2	136	485.80	366.669	299	2.80072	0.934085	19.5115	0.89521	0.68894	14065	47.0101	50	38	57
1	2	137	488.732	106.247	190	1.75179	0.821058	15.5536	0.855856	0.666667	8786	46.2421	50	35	57
1	2	138	492.338	317.287	195	1.3628	0.679385	15.757	0.933014	0.77381	5408	27.7333	29	23	34
1	3	1	10.098	359.078	102	1.21521	0.56818	11.3361	0.918919	0.713287	5508	54	58	39	68
1	3	2	14.7815	118.565	147	1.8992	0.850152	13.6809	0.88024	0.576471	5099	34.6871	37	25	44
1	3	3	16.517	274.384	176	1.49674	0.744054	14.9696	0.926316	0.647059	9866	56.0568	60	45	68
1	3	4	13	374.451	82	1.20920	0.562296	10.2179	0.88172	0.683333	4420	53.9024	56	46	64
1	3	5	23.1517	323.427	211	1.49712	0.744206	16.3907	0.905579	0.703333	9538	45.2038	47	35	55
1	3	6	22.7297	353.041	74	1.16551	0.513657	9.70668	0.880952	0.747475	4078	55.1216	56	44	66
1	3	7	23.7391	366.87	69	1.44756	0.723029	9.37302	0.873418	0.69697	3983	57.7246	59	48	70
1	3	8	34.5623	50.4478	297	2.56212	0.920687	19.4461	0.838983	0.53982	11784	39.6768	41	31	48
1	3	9	33.9742	109.832	155	1.63061	0.789875	14.0482	0.933735	0.691964	4887	31.529	33	24	39
1	3	10	44.7725	455.738	233	1.9878	0.864246	13.228	0.869103	0.667222	9369	40.2103	42	31	50
1	3	11	51.9162	275.366	191	1.67447	0.80209	15.5945	0.896714	0.636667	3954	52.1152	54	42	62
1	3	12	62.4292	263.297	219	1.22358	0.581065	16.6985	0.862205	0.608333	9759	44.5616	46	36	54
1	3	13	65.3559	171.248	213	2.19727	0.890435	16.4682	0.946667	0.806818	7486	35.1155	36	27	43
1	3	14	63.4132	488.702	121	1.69052	0.806279	12.4122	0.923664	0.75625	4267	35.2615	37	28	43
1	3	15	73.3548	80.7742	135	1.30361	0.66153	14.0402	0.922619	0.745192	9210	59.871	62	50	71
1	3	16	79	329.532	183	1.97089	0.861719	15.2648	0.915	0.72619	4967	27.1421	28	21	33
1	3	17	80.0286	183.618	210	1.64688	0.796563	16.3518	0.929204	0.718286	7257	34.5571	37	27	41
1	3	18	79.6596	291.823	141	1.25467	0.603969	13.3988	0.933775	0.783333	4430	31.7553	36	27	40
1	3	19	80.1048	310.169	128	1.39545	0.697472	12.5651	0.925373	0.751515	4607	37.1532	38	30	44
1	3	20	86.0366	103.451	168	1.55708	0.766315	14.4503	0.916201	0.889078	5296	32.2927	34	25	39
1	3	21	90.3161	139.787	174	1.67033	0.800586	14.0843	0.920635	0.654135	4690	26.954	29	21	32
1	3	22	85.1818	304.591	22	1.58172	0.774787	5.29257	0.916881	0.333333	1517	68.9545	68	64	76
1	3	23	88.5014	72.4186	43	1.85078	0.848695	7.39928	0.914894	0.671875	6443	149.837	155	105	186
1	3	24	95.9063	437.878	188	1.46558	0.731051	15.8716	0.930693	0.708767	4330	23.0319	23	18	28
1	3	25	103.775	259.664	271	2.25576	0.896369	10.5755	0.73812	0.531373	10362	38.2362	40	30	47
1	3	26	101.718	351.385	109	1.36499	0.680653	11.7806	0.923729	0.778571	4566	41.8899	44	33	52
1	3	27	100.971	341.4	105	1.68452	0.804731	11.5426	0.913043	0.673077	4538	43.219	45	34	53
1	3	28	102.731	26.3077	382	1.46201	0.729519	15.2221	0.938144	0.722222	5335	29.3132	31	24	35
1	3	29	108.742	241.381	252	1.52887	0.756126	17.9125	0.818182	0.666667	10076	39.9841	43	31	49
1	3	30	107.882	297.916	130	1.2305	0.58271	12.8555	0.928571	0.722222	4831	37.1615	37	29	46
1	3	31	117.68	139.313	128	1.2113	0.564315	12.7662	0.941176	0.761905	4503	35.1797	38	28	47
1	3	32	117.676	420.503	145	1.53776	0.759681	13.5875	0.91195	0.710784	3963	27.331	28	21	34
1	3	33	119.99	374.808	104	1.58258	0.775088	11.5073	0.912281	0.675325	4622	44.4423	45	34	55
1	3	34	124.631	465.313	160	2.13828	0.883905	14.273	0.855615	0.666667	3953	24.7063	25	19	31
1	3	35	125.541	492.442	333	1.14799	0.491128	13.0131	0.93001	0.730769	3821	28.7293	30	23	34
1	3	36	129.158	286.64	114	1.81038	0.833599	12.0478	0.919355	0.791667	4570	40.0877	42	31	49
1	3	37	135.662	301.387	80	1.7702	0.825154	10.0925	0.898876	0.714286	4315	53.9375	55	42	67
1	3	38	144.778	93.2458	216	1.26728	0.611272	16.5937	0.935065	0.75	8028	37.1667	39	30	44
1	3	39	144.088	261.538	114	1.78966	0.829327	12.0478	0.912	0.626374	4590	40.2632	42	32	49
1	3	40	147.37	232.151	119	1.2221	0.574839	12.3092	0.922481	0.772727	8226	69.1261	72	52	83
1	3	41	150.157	435.098	102	1.36069	0.678155	11.3961	0.910714	0.713287	3854	37.7843	40	31	45
1	3	42	154.435	412.167	108	1.47096	0.733333	11.7265	0.915254	0.701299	4156	38.4815	40	30	46
1	3	43	158.445	295.772	254	2.29656	0.90022	17.9836	0.84106	0.529167	9778	38.4961	40	31	48
1	3	44	159.39	30.2537	201	1.64383	0.793681	15.9975	0.931884	0.705263	6992	34.7861	36	27	42
1	3	45	159.799	272.021	144	1.23912	0.590519	13.5406	0.923077	0.738462	9195	63.8547	69	52	76
1	3	46	166.627	390.108	267	1.65549	0.796946	18.4379	0.946809	0.794643	10521	39.4045	41	29	49
1	3	47	170.118	433.126	119	1.42923	0.714457	12.3092	0.915385	0.708333	4156	34.9244	36	26	43
1	3	48	174.639	471.702	191	1.36363	0.673866	15.5945	0.915545	0.757937	8388	43.9162	45	36	52
1	3	49	175.864	132.604	154	1.35535	0.675001	14.0028	0.927711	0.733333	6793	44.1104	46	34	54
1	3	50	182.212	74.0719	153	1.41914	0.709554	13.9573	0.921687	0.75	4953	32.3725	34	26	38
1	3	51	188.486	428.514	144	2.14718	0.884928	13.5406	0.89441	0.571429	4506	31.2917	33	23	39
1	3	52	196.816	173.406	293	1.59008	0.777488	19.3147	0.904321	0.636957	21249	72.5222	74	57	89
1	3	53	194.99	340.259	288	1.78224	0.827754	19.0826	0.925566	0.733333	10278	35.9301	37	29	43
1	3	54	202.502	293.631	293	2.40846	0.909729	19.3147	0.81438	0.631466	10024	34.2116	36	26	42
1	3	55	206.054	40.4423	222	1.32324	0.654892	16.8125	0.931709	0.778947	9681	43.6081	46	34	53
1	3	56	218.937	319.989	269	1.78272	0.827856	18.5068	0.896667	0.68798	10198	37.9108	40	30	45
1	3	57	217.344	409.796	250	1.92424	0.854357	17.8412	0.816993	0.548246	9609	38.436	39	29	48
1	3	58	213.452	102.311	137	1.45643	0.727025	14.1386	0.928994	0.768608	6078	38.7134	40	31	46
1	3	59	221.224	168.737	304	1.81746	0.834019	19.674	0.87106	0.603175	20052	65.9605	70	51	81
1	3	60	224.342	36.0072	149	1.27651	0.621533	13.7736	0.93125	0.776042	4599	30.8658	33	25	36
1	3	61	228.518	451.459	159	1.38781	0.693392	14.2203	0.924419	0.719457	4908	30.8679	32	25	37
1	3	62	236.586	365.865	237	1.34179	0.666762	17.3712	0.846429	0.658333	5845	24.6624	25	18	31
1	3	63	235.433	13.5773	97	1.53429	0.758418	11.1132	0.941748	0.769841	4282	44.1443	47	32	54
1	3	64	238.177	54.8398	181	1.30302	0.641115	15.1808	0.928205	0.760504	4749	26.2376	28	21	32
1	3	65	242.074	491.788	189	1.66383	0.799233	15.5126	0.935644	0.726923	4786	25.3228	26	21	30
1	3	66	239.109	254.516	128	1.27034	0.61471	12.7662	0.914286	0.761905	5025	39.2578	41	31	45
1	3	67	265.439	301.019	285	1.7139	0.83214	19.0492	0.940594	0.652174	8867	31.1123	32	25	37
1	3	68	244.548	442.484	155	1.8373	0.838906	14.0482	0.922619	0.717593	4694	30.2839	32	25	36
1	3	69	252.391	36.7091	110	1.05333	0.314159	11.8345	0.92437	0.763889	4359	39.6273	41	32	48
1	3	70	260.993	69.3096	139	1.53453	0.856033	13.3036	0.902597	0.579167	4647	33.4317	34	26	41
1	3	71	260.8	238.867	73	1.38481	0.691768	9.77205	0.882353	0.694444	8191	109.213	109	86	132
1	3	72	260.881	465.983	59	1.60172	0.804	8.66724	0.867647	0.59596	7107	120.458	121	74	167
1	3	73	265.891	16.1091	110	1.34980	0.671725	11.8345	0.92437	0.769231	4628	42.0727	44	33	51

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1	3	74	270.921	433.627	177	1.27986	0.624112	15.0121	0.917098	0.743697	9749	55.0791	55	42	69
1	3	75	273.404	87.2443	221	1.7298	0.815964	16.7746	0.884	0.613089	9572	43.3122	45	33	53
1	3	76	273.832	345.875	208	2.53252	0.91874	16.2737	0.900297	0.619048	9810	41.1435	49	36	57
1	3	77	287.91	395.184	408	2.04761	0.872635	24.9267	0.741641	0.530435	15133	31.0102	32	24	38
1	3	78	282.431	190.639	144	1.86664	0.844395	13.5406	0.9	0.666667	7827	51.3542	56	42	68
1	3	79	288.361	256.084	249	2.48567	0.928094	17.8055	0.778125	0.523109	10446	42.1124	43	33	51
1	3	80	280.085	492.403	189	1.23762	0.589177	15.5126	0.921351	0.75	4743	25.0952	27	20	30
1	3	81	283.268	23.6071	142	1.49137	0.741889	11.9416	0.888889	0.822222	4842	43.2321	47	26	59
1	3	82	286.84	279.133	393	1.2224	0.575125	19.3147	0.851293	0.820728	13463	45.9488	47	36	56
1	3	83	286.917	64.0877	114	1.53491	0.759772	12.0478	0.890425	0.690909	4589	40.2544	40	31	49
1	3	84	291.161	204.621	124	1.51011	0.749327	12.5651	0.939394	0.826667	6013	48.4919	51	39	57
1	3	85	294.434	349.69	129	1.33034	0.659521	12.8159	0.902098	0.716667	6258	48.5116	51	39	58
1	3	86	299	473.056	234	1.32743	0.657635	17.2609	0.83871	0.724458	8552	36.547	38	28	44
1	3	87	295.08	329.593	113	1.60776	0.783031	11.9940	0.896625	0.620879	5052	44.708	47	36	54
1	3	88	305.782	148.492	238	1.37652	0.687198	17.4078	0.929488	0.695906	10710	45	41	35	54
1	3	89	308.422	376.07	128	1.92602	0.85465	12.7662	0.927536	0.790123	4686	36.6094	38	28	45
1	3	90	310.653	41.0155	323	1.64555	0.794168	20.2795	0.828205	0.621154	9695	30.0155	29	21	43
1	3	91	317.211	458.409	473	1.49285	0.742486	24.5406	0.852257	0.615885	16175	34.1946	35	25	42
1	3	92	310.3	258.74	100	1.66277	0.798943	11.2838	0.900901	0.714286	4835	48.75	51	38	58
1	3	93	316.394	172.631	109	1.70657	0.810332	11.7806	0.939655	0.807407	4793	43.9511	44	34	54
1	3	94	319.03	158.242	99	1.44078	0.719907	11.2272	0.908257	0.692308	4732	47.799	49	39	58
1	3	95	320.597	216.664	134	1.40176	0.700769	13.0619	0.930556	0.812121	4915	36.4191	37	28	46
1	3	96	330.547	247.986	139	1.17331	0.523071	13.3314	0.924647	0.743736	5010	36.0432	36	24	46
1	3	97	337.579	327.423	274	2.09771	0.874442	18.5755	0.808155	0.4775	10398	33.431	40	29	48
1	3	98	333.177	395.24	192	1.71139	0.81299	15.6353	0.964178	0.473491	10744	51.3542	54	41	66
1	3	99	331.564	104.017	172	1.40901	0.703419	14.7984	0.924731	0.751386	9962	51.8073	59	48	69
1	3	100	338.308	360.632	133	1.15587	0.501454	13.0131	0.923611	0.791667	5765	39.406	40	31	47
1	3	101	342.452	380.048	144	1.40379	0.701538	12.5651	0.911519	0.681319	5215	42.3565	45	33	51
1	3	102	351.184	235.709	179	1.78771	0.829014	15.0941	0.899191	0.611111	5618	28.3467	29	22	35
1	3	103	349.87	241.407	141	1.21395	0.544840	14.3115	0.961059	0.815441	5106	31.3143	32	24	38
1	3	104	355.573	192.699	204	1.75463	0.8717	16.1953	0.919463	0.64375	7106	37.8417	38	29	47
1	3	105	367.143	124.473	357	1.5789	0.773864	21.3701	0.798658	0.684528	19382	84.7813	51	40	67
1	3	106	365.197	79.6384	277	1.28105	0.629539	15.0121	0.931579	0.747497	4016	27.769	29	21	33
1	3	107	364.781	79.4298	114	1.04945	0.354947	12.0478	0.912	0.710749	4537	39.7467	40	29	50
1	3	108	364.517	350.4	120	1.4471	0.722819	12.3608	0.941882	0.8	4489	39.035	40	32	46
1	3	109	368.913	285.978	46	1.36168	0.679096	7.65304	0.884615	0.638889	4706	147.322	151	117	181
1	3	110	380.066	330.11	127	1.40286	0.421704	12.7162	0.913669	0.751479	4777	37.6142	40	30	46
1	3	111	381.023	50.5496	131	1.54783	0.76328	12.9149	0.916088	0.744318	4369	33.3511	34	27	40
1	3	112	384.055	301.782	165	1.49253	0.74236	14.1943	0.953757	0.808824	9802	59.4061	62	48	70
1	3	113	386.369	406.795	195	2.07424	0.876114	15.757	0.933014	0.770751	5143	26.3744	28	19	33
1	3	114	390.813	384.067	134	1.35801	0.676576	13.0619	0.937063	0.812121	4920	36.7164	39	30	43
1	3	115	402.104	371.906	202	1.96693	0.861118	16.0373	0.918102	0.655844	5475	27.104	28	22	33
1	3	116	402.82	214.787	239	1.7078	0.810637	17.4443	0.895131	0.663889	13837	57.8954	58	43	74
1	3	117	407.701	469.368	117	1.49392	0.742919	12.2053	0.936	0.75974	4368	37.2735	39	29	46
1	3	118	412.591	72.0269	186	2.25551	0.896771	15.389	0.920792	0.673913	4700	25.2688	27	19	31
1	3	119	420.651	404.54	137	1.87173	0.845318	13.2073	0.913333	0.652381	6143	44.9854	48	36	54
1	3	120	421.21	439.79	100	1.74133	0.818664	11.2838	0.884956	0.666667	4125	41.25	42	32	51
1	3	121	421.597	220.842	47	1.77433	0.826053	9.23618	0.917808	0.697917	4008	59.8709	60	47	73
1	3	122	428.003	326.659	132	1.37134	0.684287	12.9641	0.923077	0.781065	4590	34.7727	35	27	43
1	3	123	428.429	448.696	112	1.35339	0.673829	11.9416	0.92562	0.8	4511	40.2768	42	33	48
1	3	124	440.479	30.7668	193	1.83016	0.837524	15.6759	0.919048	0.70696	4646	24.0725	25	19	29
1	3	125	441.736	399.443	248	1.39449	0.696963	17.6979	0.935361	0.719298	10094	41.0325	42	32	50
1	3	126	439.733	202.893	75	1.92194	0.85398	9.77205	0.872093	0.568182	4292	57.2267	61	44	71
1	3	127	439.909	324.937	44	1.33845	0.664673	7.48482	0.956522	0.785714	6863	155.977	161	120	193
1	3	128	444.902	135.214	173	1.15723	0.503268	14.8415	0.920213	0.720833	4938	28.5434	30	22	35
1	3	129	445.937	448.147	95	1.25669	0.605633	10.9981	0.904762	0.730769	4531	47.6947	48	36	59
1	3	130	444.353	188.412	34	1.24146	0.592169	6.57952	0.85	0.708333	5614	165.118	176	108	219
1	3	131	447.062	79.8769	330	1.11577	0.443558	12.8655	0.928571	0.77381	4603	35.4077	38	28	42
1	3	132	449.534	47.0451	333	1.06744	0.349099	13.0133	0.904762	0.678571	4603	35.2105	36	27	42
1	3	133	450.026	354.36	114	1.28391	0.627186	12.0478	0.942149	0.74026	4701	41.2368	42	32	51
1	3	134	460.248	303.075	226	3.00305	0.942929	16.9633	0.784722	0.627221	8764	38.7788	31	24	42
1	3	135	451.634	476.634	71	1.27897	0.623433	9.50189	0.910256	0.788889	4160	58.5915	60	48	70
1	3	136	463.959	250.088	148	1.36367	0.679888	13.7273	0.936709	0.758974	5502	37.1757	38	29	44
1	3	137	466.043	56.1739	23	1.31472	0.649198	5.41152	0.851052	0.657143	2634	114.522	118	89	149
1	3	138	465.3	63.85	20	1.20783	0.555733	5.04827	0.833333	0.666667	2430	121.5	132	87	154
1	3	139	474.931	370.517	87	1.37483	0.668253	10.5248	0.878788	0.669231	5493	63.1379	66	51	77
1	3	140	478.665	164.937	158	1.39125	0.695242	14.1835	0.929412	0.77451	8738	55.3038	56	42	70
1	3	141	475.295	102.409	44	1.52203	0.751875	7.48482	0.916667	0.698413	6727	152.886	156	111	188
1	3	142	482.725	813.554	204	1.77691	0.826609	16.1165	0.898678	0.8	9379	45.9755	48	35	59
1	3	143	485.172	58.085	294	1.84407	0.721431	19.3477	0.869822	0.786096	17665	60.085	62	47	72
1	3	144	482.755	453.941	102	1.94739	0.858085	11.3961	0.902655	0.708333	5666	55.549	56	37	71
1	3	145	480.108	483.45	120	1.77476	0.826144	12.3608	0.909091	0.681818	4250	35.4833	36	27	44
1	3	146	483.899	116.449	158	1.2289	0.501235	14.1835	0.918605	0.705357	4655	29.462	30	22	36
1	3	147	496.56	345.393	238	1.53534	0.758803	17.4788	0.792553	0.627368	9905	33.2383	35	25	41
1	3	148	489.322	41.1774	87	1.17086	0.520149	10.5248	0.887755	0.725	3815	43.8506	46	35	52
1	3	149	495.5	489.606	128	1.50512	0.747379	12.7662	0.934307	0.8	4444	34.7188	35	25	43

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1	3	150	491.606	286.066	35	1.13938	0.541305	5.67558	0.965946	0.833333	3545	101.286	105	89	117
1	3	151	497.438	76.3769	330	2.04206	0.871891	12.6655	0.902778	0.637255	4553	35.0231	36	26	43
1	3	152	499.836	144.308	128	1.24075	0.591964	12.7662	0.920863	0.111111	4856	37.9375	39	30	46
1	3	153	504.436	285.482	39	1.4304	0.715017	7.04673	0.906977	0.8125	3680	94.359	96	79	113
1	4	1	20	122.671	152	1.3561	0.675449	13.9116	0.923212	0.730769	4832	31.7895	33	25	39
1	4	2	22.2081	72.3198	197	1.33473	0.662327	15.8376	0.929245	0.72963	9490	48.1726	50	37	59
1	4	3	22.1391	148.063	335	1.35737	0.676433	12.1005	0.92	0.746753	4511	39.2261	40	30	48
1	4	4	23.2778	287.022	137	1.66669	0.800492	13.2073	0.931973	0.805882	6042	44.1022	47	35	53
1	4	5	26.6026	348.245	151	1.68695	0.804842	13.8658	0.909639	0.693074	7260	48.0795	50	38	57
1	4	6	28.0294	162.559	102	1.28739	0.626493	11.3961	0.93578	0.784615	4532	44.4314	45	36	53
1	4	7	36.734	487.684	282	2.28224	0.898894	18.9487	0.817391	0.592437	9658	34.2482	35	26	43
1	4	8	39.2951	188.77	183	2.36982	0.906608	15.2644	0.905941	0.571875	4775	26.0929	27	19	33
1	4	9	45.5199	217.316	377	2.90967	0.939086	21.9092	0.821351	0.419355	15415	40.8886	35	26	60
1	4	10	40.3109	306.084	119	1.46635	0.731385	12.3092	0.929688	0.793333	4745	39.8739	41	29	49
1	4	11	49.0224	124.27	259	2.13833	0.883911	18.1595	0.799383	0.535124	10090	38.9575	41	30	48
1	4	12	53.6559	76.3118	186	1.22682	0.579108	15.389	0.944162	0.781513	9469	50.9086	53	42	60
1	4	13	61.75	496.487	156	1.3807	0.689518	14.0935	0.928571	0.784706	6401	41.0321	42	29	54
1	4	14	61.8638	110.813	96	1.5645	0.769055	13.0558	0.897196	0.671329	4500	46.875	48	38	56
1	4	15	70.4453	190.465	172	2.30563	0.901067	14.7986	0.900524	0.532508	5838	33.9419	35	27	41
1	4	16	69.6914	97.7778	81	1.43608	0.717712	10.1554	0.9	0.75	4431	54.7037	58	43	65
1	4	17	87.0529	100.461	102	1.86101	0.843364	11.3961	0.894737	0.708333	4364	42.7843	46	33	52
1	4	18	89.9259	68.0148	108	1.34834	0.493599	11.7265	0.915254	0.755245	4387	40.6201	43	33	46
1	4	19	98.8842	183.474	190	2.31245	0.901662	15.5536	0.92233	0.688406	7736	40.7158	42	32	49
1	4	20	98.6446	473.239	251	1.39113	0.495175	17.8769	0.933086	0.747024	10787	42.9761	46	34	51
1	4	21	96.8018	333.613	111	1.68667	0.805207	11.8882	0.917355	0.74	4686	42.2162	44	33	51
1	4	22	96.6612	405.571	121	1.31338	0.648287	12.4122	0.914667	0.785714	4595	37.9752	41	29	46
1	4	23	98.7228	41.5248	101	1.22405	0.576693	11.3401	0.90991	0.765152	4325	42.8218	44	33	52
1	4	24	100.188	110.136	176	1.48194	0.738011	14.9696	0.924316	0.739496	5059	28.7443	29	22	34
1	4	25	101.765	300.07	115	1.86325	0.843776	12.1005	0.884615	0.588744	4560	39.6522	41	31	49
1	4	26	108.133	141.783	135	1.06071	0.333452	13.1106	0.918367	0.781758	4866	36.0444	36	28	45
1	4	27	118.875	443.208	48	2.28411	0.839069	7.81764	0.774194	0.623377	5782	120.458	127	74	168
1	4	28	124.705	39.2679	112	1.93298	0.85896	11.9416	0.88189	0.622222	4383	39.1339	41	31	47
1	4	29	123.518	339.373	110	1.55515	0.769275	11.8345	0.92437	0.705128	4488	40.8	43	33	49
1	4	30	123.016	410.445	128	1.20716	0.560153	12.7662	0.907801	0.761905	4694	36.6719	37	29	45
1	4	31	130.844	80.248	125	1.35859	0.676922	12.6157	0.925926	0.757576	4636	37.088	38	28	45
1	4	32	135.846	109.582	182	1.19473	0.551436	15.2227	0.928571	0.764706	5123	28.1484	29	22	34
1	4	33	140.518	124.895	164	1.68308	0.804354	14.4503	0.921348	0.759259	4956	30.2195	31	24	36
1	4	34	141.5	164.779	104	1.25753	0.606338	11.5073	0.912281	0.722222	4404	42.3462	44	35	50
1	4	35	151.336	265.602	128	1.35544	0.675056	12.7662	0.907801	0.711111	4698	36.7031	39	30	44
1	4	36	154.318	473.529	157	1.65981	0.798134	14.1386	0.928994	0.654167	4848	30.879	31	25	38
1	4	37	153.259	431.776	116	1.73133	0.816326	12.152	0.892308	0.644444	4545	39.181	38	29	49
1	4	38	157.479	292.536	140	1.53578	0.758963	13.3512	0.915033	0.729167	4828	34.4857	35	27	43
1	4	39	160.654	263.442	156	1.03336	0.252051	14.0935	0.923077	0.742857	9609	61.5962	64	49	73
1	4	40	163.455	141	121	1.71923	0.813636	12.4122	0.896256	0.664835	4553	37.6281	39	29	44
1	4	41	164.635	45.5079	63	1.72672	0.815232	8.95623	0.913043	0.63	7016	111.365	116	91	136
1	4	42	168.561	331.13	82	1.63975	0.392517	10.2179	0.87234	0.621212	4075	49.6951	52	40	60
1	4	43	167.286	389.735	98	1.61962	0.786627	11.1704	0.882883	0.7	4307	43.949	46	35	52
1	4	44	171.548	93.6129	341	2.5791	0.921772	20.8369	0.833741	0.615833	14949	43.8387	45	33	55
1	4	45	170.747	210.678	87	1.21387	0.566866	10.5248	0.915789	0.790909	4778	54.9195	56	43	65
1	4	46	172.538	433.169	330	1.4418	0.72038	12.8655	0.915493	0.714286	4816	37.0462	37	29	46
1	4	47	182.634	395.457	81	1.03532	0.250957	10.1554	0.94186	0.81	4816	59.4568	60	45	71
1	4	48	188.789	451.247	194	1.86937	0.844891	15.7165	0.941748	0.734848	5054	26.0515	27	20	32
1	4	49	197.623	222.729	579	1.57599	0.772905	27.1515	0.772	0.486555	24028	41.4991	42	31	53
1	4	50	207.162	275.591	154	1.34856	0.670916	14.0028	0.933333	0.802083	7416	48.1558	51	37	56
1	4	51	208.366	19.4718	142	1.39993	0.699817	13.4462	0.922078	0.739583	4462	31.4225	32	24	39
1	4	52	216.594	153.421	254	1.67351	0.801835	17.9834	0.897527	0.636591	12587	49.5551	51	37	62
1	4	53	216.26	292.683	123	1.23562	0.587384	12.5143	0.911111	0.732143	7499	60.9675	63	51	73
1	4	54	223.36	332.144	125	1.10482	0.425139	12.6157	0.93985	0.801282	4674	37.392	38	29	44
1	4	55	224.5	17	8	1.19523	0.547723	3.19154	0.8	0.666667	82	10.75	11	6	14
1	4	56	235.095	181:581	241	2.2866	0.895301	17.5172	0.836806	0.617949	7444	30.888	32	22	39
1	4	57	230.172	402.352	128	1.20362	0.556529	12.7662	0.907801	0.65641	5212	40.7188	41	31	50
1	4	58	236.048	79.3832	167	1.40843	0.704192	14.5819	0.922652	0.755656	4581	27.4311	29	22	33
1	4	59	238.949	345.641	117	1.271	0.617231	12.2053	0.928571	0.75974	4614	39.453	41	30	48
1	4	60	241.222	378.652	81	1.37505	0.686378	10.1554	0.89011	0.75	6038	74.5432	78	59	87
1	4	61	252.197	247.61	385	3.61941	0.961075	22.1408	0.819143	0.49359	11357	29.4987	31	23	36
1	4	62	259.604	354.2	95	1.16027	0.507128	10.9981	0.88785	0.719697	4295	45.2105	47	38	54
1	4	63	268.422	143.438	315	1.63168	0.837859	20.0267	0.791457	0.616275	9714	30.8381	33	24	37
1	4	64	272.363	234.879	256	1.57039	0.771041	18.0541	0.911032	0.711111	8749	34.1758	34	28	42
1	4	65	271.957	343.086	93	1.42486	0.71428	10.8817	0.894231	0.704545	4324	46.4946	47	36	57
1	4	66	275.955	27.2211	199	1.5295	0.756659	15.9177	0.925581	0.690972	4521	22.7186	24	18	27
1	4	67	273.804	327.134	97	1.98725	0.864166	11.1132	0.873874	0.573964	4365	45	46	36	55
1	4	68	278.578	102.532	109	1.44495	0.730775	11.7806	0.886179	0.698718	5405	49.5872	50	37	63
1	4	69	279.534	205.402	103	1.37098	0.684081	11.4518	0.887931	0.72028	4411	42.8252	44	32	52
1	4	70	288.613	488.525	261	2.99776	0.942721	18.2295	0.852941	0.466071	9287	35.6207	36	27	46
1	4	71	283.109	451.764	55	1.49925	0.8085	8.36828	0.873016	0.611111	6359	115.618	120	91	142
1	4	72	290.576	78.3273	165	1.96088	0.860189	14.4943	0.932203	0.75	5053	30.6242	32	24	38

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1	4	73	308.012	29.4471	170	1.2847	0.62778	14.7123	0.309091	0.674603	4599	23.0471	28	21	33
1	4	74	306.881	474.526	126	1.97041	0.861446	12.666	0.893617	0.6	4490	35.5556	38	28	43
1	4	75	310.799	253.469	299	1.48785	0.744498	19.5115	0.911315	0.479545	20353	48.0702	71	55	81
1	4	76	323.332	189.974	248	1.56218	0.768264	18.4774	0.847314	0.42037	9821	34.6455	39	27	44
1	4	77	320.089	159.319	169	1.58065	0.774437	14.4489	0.914474	0.722222	7397	43.7692	44	35	52
1	4	78	321.261	132.151	119	1.63078	0.789119	12.3092	0.919488	0.708142	4478	39.3109	41	33	48
1	4	79	331.813	442.724	123	1.51786	0.1523	12.5143	0.939931	0.82	4425	37.6018	39	30	44
1	4	80	341.041	46.986	358	1.87433	0.845386	21.3499	0.813838	0.581849	15308	42.7474	65	37	51
1	4	81	340.704	254.099	233	2.05938	0.874056	17.224	0.924603	0.716923	6464	29.1532	33	23	35
1	4	82	346.781	107.539	256	2.95123	0.940843	19.0541	0.864865	0.463788	7926	30.9409	33	24	38
1	4	83	346.119	140.637	160	1.20679	0.559717	14.211	0.914286	0.714286	5934	37.0415	39	29	45
1	4	84	350.881	37.457	151	1.13385	0.473336	13.8458	0.909439	0.719048	5405	35.7547	36	28	43
1	4	85	351.833	472.913	138	1.57443	0.732335	13.2555	0.92	0.737888	4317	34.1812	35	28	41
1	4	86	370.358	449.906	117	1.27551	0.420782	12.2053	0.934063	0.73574	4418	33.453	41	33	43
1	4	87	374.471	140.044	225	1.49307	0.744984	16.9251	0.929752	0.755304	9474	41.4472	45	33	54
1	4	88	376.337	504.93	86	1.25777	0.602318	10.4442	0.905263	0.781818	3841	44.4478	48	34	53
1	4	89	389.695	89.1484	190	1.2873	0.429721	15.5534	0.917467	0.715091	6740	35.4737	37	28	43
1	4	90	397.79	245.253	186	1.85957	0.850216	15.349	0.920797	0.441288	8465	45.5108	47	35	54
1	4	91	391.993	57.8739	113	1.61906	0.78444	11.8882	0.925	0.822222	4218	38	39	30	45
1	4	92	409.164	203.288	229	1.69095	0.806392	17.0755	0.944281	0.800699	9587	42.3013	44	34	51
1	4	93	404.844	33.6234	77	1.36656	0.681557	8.90149	0.930617	0.875	1522	97.6883	97	79	117
1	4	94	419.253	248.542	356	1.74772	0.820132	21.2902	0.872549	0.585528	12819	36.0084	37	28	44
1	4	95	408.800	14.7345	73	1.35563	0.675165	9.64088	0.890244	0.737374	7784	106.63	111	84	127
1	4	96	414.795	347.565	161	2.1258	0.931079	14.3375	0.894444	0.526144	5842	36.2857	37	27	46
1	4	97	414.418	47.1493	67	1.17547	0.525413	9.23618	0.905405	0.744444	4024	60.0597	62	47	71
1	4	98	413.019	491	107	2.96142	0.936945	11.672	0.829457	0.66875	3863	36.1589	34	25	49
1	4	99	420.848	178.549	144	1.4175	0.708939	13.5406	0.917197	0.75	4490	31.1806	33	24	37
1	4	100	423.104	104.057	106	1.43449	0.716964	11.6174	0.898305	0.627219	4077	38.4623	39	31	46
1	4	101	426.239	160.553	188	1.43937	0.719252	15.4716	0.930693	0.734375	8843	47.0372	49	36	58
1	4	102	425.926	63.8589	163	1.84936	0.938199	14.4062	0.835897	0.679167	8227	50.4724	48	35	65
1	4	103	438.319	109.233	116	1.445	0.72186	12.153	0.913386	0.70303	4171	35.9569	38	27	45
1	4	104	445.092	158.708	120	1.48149	0.73782	12.3608	0.916031	0.710059	4342	36.35	38	28	43
1	4	105	448.698	438.14	172	1.7764	0.876499	14.7986	0.924731	0.632353	4888	28.4146	30	21	35
1	4	106	455.915	28.6154	130	1.30643	0.643502	12.8655	0.928571	0.787879	6559	50.4518	53	40	61
1	4	107	462.451	83.2254	142	1.20737	0.560366	13.4442	0.928105	0.788889	6157	43.3592	46	35	52
1	4	108	470.954	328.27	152	2.62376	0.92452	13.9816	0.894118	0.5	4143	27.2566	30	20	33
1	4	109	465.05	442.9	20	1.08287	0.383662	5.04627	0.469565	0.8	58	2.9	3	1	4
1	4	110	473.657	318.841	245	1.19531	0.743182	17.6619	0.935115	0.720588	9593	39.1551	39	30	50
1	4	111	479.737	255.395	152	1.89311	0.849105	13.9116	0.921212	0.666667	5655	37.2039	39	29	45
1	4	112	485.175	433.833	309	1.50055	0.715576	19.8351	0.830615	0.588571	14140	45.7605	48	35	56
1	4	113	488.297	19.5034	145	1.7151	0.812432	13.5875	0.900621	0.641321	4612	31.8069	33	22	41
1	5	1	12.1124	81.2878	169	1.75774	0.822397	14.6689	0.83418	0.625926	5289	31.2959	33	24	38
1	5	2	21.6842	157.041	114	1.32558	0.658429	12.0478	0.890625	0.690909	4450	39.0351	41	30	47
1	5	3	26.5433	251.717	127	1.39877	0.698164	12.7162	0.913669	0.697802	4420	34.8031	35	27	42
1	5	4	33.697	139.273	132	1.66927	0.800701	12.9611	0.923077	0.6875	4555	34.5076	36	27	42
1	5	5	31.8739	216.532	111	1.64226	0.793234	11.8892	0.909836	0.770833	4359	39.2703	42	29	48
1	5	6	39.4234	413.721	222	1.28116	0.625105	16.8125	0.944681	0.687307	9624	43.3514	45	34	53
1	5	7	42.3592	301.33	103	1.85131	0.841564	11.4518	0.880342	0.64375	4366	42.3883	44	32	54
1	5	8	40.6712	471.411	222	1.66922	0.800606	16.8125	0.925	0.776224	9583	43.1667	44	33	53
1	5	9	46.598	35.2684	159	1.47235	0.733967	15.9177	0.934272	0.731618	9167	46.0653	48	37	54
1	5	10	52.8175	226.248	126	1.33284	0.661326	12.666	0.919708	0.763636	4550	36.1111	38	29	44
1	5	11	56.0102	81.1371	197	1.15958	0.506263	15.8376	0.938095	0.772549	8944	45.401	47	37	55
1	5	12	55.3209	315.187	134	1.09487	0.407181	13.0619	0.920556	0.736264	4700	35.0718	37	28	42
1	5	13	58.7305	465.979	141	1.46864	0.732373	13.3988	0.927632	0.671429	4519	32.0496	34	25	39
1	5	14	59.9231	102.546	130	1.06579	0.34589	12.8655	0.915493	0.769231	4593	35.3308	37	29	42
1	5	15	59.8505	253.991	107	1.52309	0.754275	11.672	0.884298	0.633136	4346	40.6168	42	33	50
1	5	16	77.2771	393.892	166	1.68073	0.80374	14.5381	0.948571	0.794258	8121	48.9217	52	40	53
1	5	17	75.5	213	106	1.52953	0.75667	11.6174	0.898305	0.688312	4366	41.1887	42	31	50
1	5	18	83.3667	139.839	180	1.37201	0.684667	15.1388	0.923077	0.714286	8487	47.15	49	37	57
1	5	19	78.669	245.397	142	1.7102	0.811229	13.4462	0.922078	0.759358	4639	32.669	33	25	40
1	5	20	80.7353	114.421	102	1.17957	0.530312	11.3961	0.910714	0.772727	4258	41.7451	44	34	49
1	5	21	85.9789	374.095	95	1.41567	0.707832	10.9981	0.904762	0.644336	4181	44.0105	45	36	54
1	5	22	89.9514	349.229	144	1.57597	0.772499	13.5406	0.917197	0.685714	4691	32.5764	34	26	39
1	5	23	89.4218	469.204	147	1.53767	0.759648	13.6809	0.907407	0.65625	4770	32.449	35	25	39
1	5	24	122.446	39.3708	178	1.24309	0.594028	15.0545	0.941799	0.744667	9293	52.2079	56	42	61
1	5	25	127.254	244.36	114	1.10719	0.429255	12.0478	0.926829	0.730769	4409	38.6754	40	31	47
1	5	26	143.749	122.979	233	2.33101	0.903306	17.224	0.906615	0.557416	9711	41.4781	43	34	50
1	5	27	185.205	178.486	144	1.42193	0.710922	13.5406	0.923077	0.75	5604	38.9167	41	32	45
1	5	28	190.814	79.4602	113	1.25741	0.60205	11.9948	0.918699	0.733766	4431	39.2124	40	31	47
1	5	29	193.435	196.613	168	1.65526	0.796082	14.6255	0.913043	0.65625	8079	48.0893	51	38	58
1	5	30	193.198	360.657	108	1.62453	0.71319	11.7265	0.915254	0.771429	4344	40.2222	41	31	50
1	5	31	205.619	335.608	97	1.53037	0.756983	11.1137	0.866071	0.421795	4188	43.1753	44	34	53
1	5	32	210.041	95.5338	148	1.67721	0.802814	13.7273	0.886226	0.689683	4698	31.7432	32	24	39
1	5	33	211.803	25.4	180	1.8042	0.847541	15.1388	0.913704	0.692308	8908	49.4889	53	40	58
1	5	34	233.703	73.9248	133	1.84276	0.83995	13.0131	0.93007	0.71123	4560	34.2857	35	26	41
1	5	35	243.053	255.215	685	1.7752	0.82624	29.5325	0.710581	0.465986	29016	42.3591	44	34	52

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1	5	36	244.883	44.7186	334	1.70565	0.810104	20.6219	0.885942	0.703158	15684	46.988	49	38	55
1	5	37	246.434	115.89	145	1.57559	0.772771	13.5875	0.917722	0.691115	4689	32.3379	33	25	39
1	5	38	245.763	368.532	173	1.6535	0.725714	14.8415	0.905759	0.686500	9292	53.711	56	44	64
1	5	39	259.865	297.801	148	1.74551	0.819627	13.7273	0.907975	0.869683	6158	41.6081	44	33	50
1	5	40	265.892	35.5	186	1.69253	0.806796	15.389	0.925373	0.645833	7714	41.4731	44	34	50
1	5	41	267.611	474.84	288	1.69734	0.808018	19.1492	0.857143	0.626087	12451	43.2326	45	33	53
1	5	42	276.033	256.171	152	1.66026	0.798253	13.9116	0.91018	0.703701	6365	41.875	44	34	49
1	5	43	288.878	481.766	123	1.8018	0.831849	12.5143	0.91791	0.803922	4486	36.4735	38	29	43
1	5	44	295.075	447.753	146	1.6856	0.805009	13.6343	0.918239	0.737374	5724	39.2055	40	30	46
1	5	45	300.664	879.541	122	1.79398	0.830231	12.4634	0.917293	0.797386	4489	36.7951	39	30	43
1	5	46	318.606	113.228	254	1.95525	0.859316	17.9834	0.830065	0.574661	10071	39.4528	42	31	47
1	5	47	314.634	410.758	131	1.27616	0.621266	12.9149	0.89726	0.668367	7479	60.145	63	48	73
1	5	48	331.614	418.425	391	1.44851	0.732318	22.3123	0.807851	0.55698	14741	47.9309	47	36	62
1	5	49	333.399	87.0629	143	1.88512	0.847704	13.4935	0.910878	0.752632	4653	32.5385	34	27	38
1	5	50	346.493	100.553	219	2.21567	0.892357	16.6985	0.943966	0.736364	5193	23.7123	24	19	29
1	5	51	348.328	442.77	374	1.41208	0.706034	14.8843	0.935484	0.725	6367	36.592	37	30	44
1	5	52	356.336	138.177	232	1.8871	0.848052	17.187	0.939271	0.659091	5191	22.375	24	18	27
1	5	53	363.332	178.751	265	1.66537	0.799621	18.3687	0.886288	0.752841	11544	43.5623	45	36	52
1	5	54	368.601	52.3114	228	1.73712	0.817686	17.0382	0.919355	0.633333	9439	41.3991	43	33	50
1	5	55	372.972	19.7938	713	1.41274	0.706369	16.4682	0.938326	0.747268	9210	43.2394	44	32	54
1	5	56	374.753	117.607	150	1.29951	0.638622	13.8198	0.943396	0.78125	4877	32.5133	34	27	38
1	5	57	374.303	311.178	152	1.35038	0.694272	13.9116	0.921212	0.77551	4807	31.625	32	26	38
1	5	58	377.8	326.522	115	1.45456	0.726189	12.1005	0.912698	0.737179	4437	38.5826	40	30	46
1	5	59	378.921	363.124	266	1.84987	0.841194	18.4033	0.889632	0.738889	9883	37.1541	39	29	44
1	5	60	384.396	268.143	230	2.08748	0.872617	17.1127	0.916335	0.575	8281	36.0013	38	29	44
1	5	61	385.282	492.842	202	1.86235	0.84372	16.0373	0.935185	0.765152	8567	42.4109	45	34	51
1	5	62	410.504	429.727	139	1.74983	0.820613	13.3034	0.908497	0.620536	4291	30.8705	32	25	36
1	5	63	411.49	466.68	259	1.41752	0.708752	18.1595	0.943255	0.770833	9626	37.166	38	30	45
1	5	64	422.424	175.104	328	1.81488	0.834505	20.4358	0.81592	0.585714	10428	31.7957	35	25	38
1	5	65	443.121	154.822	353	1.61841	0.788264	21.2003	0.822844	0.613913	12182	34.5099	36	25	42
1	5	66	440.54	90.5832	202	1.33459	0.686118	16.0373	0.897778	0.701389	9021	44.6584	47	35	54
1	5	67	454.759	375.696	257	2.97619	0.961862	18.0893	0.853532	0.694231	9329	34.2996	38	27	45
1	5	68	452.566	444.495	136	1.59497	0.779044	13.159	0.92517	0.708333	4269	31.3893	33	25	38
1	5	69	464.612	335.218	294	1.32695	0.657325	19.3477	0.830508	0.608496	15405	52.398	55	40	63
1	5	70	460.918	261.667	326	1.6291	0.789433	20.3734	0.91573	0.686316	18203	55.8374	58	45	66
1	5	71	465.042	44.1354	96	1.9317	0.855575	11.0558	0.90566	0.623377	3616	37.6667	39	29	46
1	5	72	471.12	211.108	251	1.78261	0.827833	17.8769	0.922794	0.747024	9386	37.3944	39	30	45
1	5	73	466.162	66.9279	111	1.23308	0.585076	11.8882	0.909836	0.720779	4120	37.1171	39	29	45
1	5	74	472.917	140.042	168	1.49961	0.745139	14.6255	0.908108	0.458824	5693	33.8869	36	26	41
1	5	75	472.007	452	146	1.38385	0.691246	13.6343	0.929936	0.748718	4402	30.1507	32	25	36
1	5	76	480.38	141.804	158	1.088	0.393983	14.1835	0.923977	0.752381	6223	39.3861	43	31	46
1	5	77	493.022	324.5	228	1.49614	0.743814	17.0382	0.926829	0.705882	9361	42.8114	45	31	53
1	5	78	480.088	485.956	133	1.31461	0.649128	11.9948	0.918699	0.733766	4219	37.3363	38	30	44
1	5	79	484.238	427.325	126	1.78479	0.828297	12.666	0.926471	0.642857	4172	33.1111	35	27	40
1	5	80	488.556	365.677	124	1.60356	0.781338	12.5651	0.898551	0.632653	4430	35.7258	37	28	44
1	5	81	492.935	486.839	124	1.7483	0.820264	12.5651	0.925373	0.810458	4449	35.879	37	31	41
1	5	82	500.688	443.375	112	1.26825	0.615051	11.9416	0.896	0.717949	4260	38.0357	40	29	46
1	6	1	13.3247	32.0206	194	1.80457	0.832413	15.7165	0.889908	0.577381	5416	27.9175	30	20	35
1	6	2	10.9224	265.724	116	1.37589	0.686846	12.153	0.920635	0.753247	4490	34.7069	35	29	48
1	6	3	18.9744	434.487	117	1.20659	0.559577	12.2053	0.914063	0.692308	3922	33.5214	34	26	41
1	6	4	27.2042	342.218	142	1.54653	0.76282	13.4462	0.934211	0.759358	4693	33.0493	35	26	40
1	6	5	27.2273	359.023	220	1.08045	0.276128	16.7366	0.92437	0.718954	6697	39.5318	41	31	48
1	6	6	33.1343	143.623	499	3.10618	0.94676	25.2061	0.799679	0.567015	24853	49.8056	46	36	68
1	6	7	42.9868	316.072	152	1.23866	0.590105	13.9116	0.915663	0.730769	4796	31.5526	32	25	39
1	6	8	46.016	262.755	188	2.09621	0.878875	15.4716	0.890995	0.580247	8627	45.8883	46	36	58
1	6	9	55.7664	473.964	214	1.83381	0.838233	16.5068	0.918455	0.715719	9014	42.1215	44	33	51
1	6	10	54.8431	308.078	102	1.43966	0.719387	11.3961	0.886957	0.728571	4113	40.3235	42	31	49
1	6	11	63.0966	31.486	321	1.12537	0.45869	20.2166	0.865229	0.663223	16634	51.8193	53	38	65
1	6	12	59.014	78.3497	143	1.91324	0.857532	13.4935	0.928571	0.722222	4232	29.5944	31	24	36
1	6	13	60.8814	420.223	166	1.50226	0.746252	14.5381	0.937853	0.751131	4349	24.1988	27	20	32
1	6	14	65.4294	230.122	197	1.39788	0.698748	15.8376	0.938095	0.781766	6672	41.0203	45	34	55
1	6	15	66.0072	459.725	138	1.08544	0.388889	13.2555	0.926174	0.758242	4182	30.3043	32	23	36
1	6	16	74	100.5	118	1.41026	0.70512	12.2573	0.921875	0.786667	4250	36.0169	37	29	43
1	6	17	82.3861	153.073	316	2.04526	0.872317	20.0585	0.890141	0.681034	15035	47.5791	46	34	63
1	6	18	74.5	76.3	140	1.2011	0.553925	13.3512	0.927152	0.777778	4409	31.4929	33	26	37
1	6	19	75.0336	296.849	119	1.49162	0.741988	12.3092	0.922481	0.721712	4261	35.8067	37	28	44
1	6	20	96.0563	217.457	129	1.11079	0.435352	12.8159	0.934783	0.763326	4179	32.3953	34	25	39
1	6	21	96.313	242.878	115	1.36483	0.680562	12.1005	0.912698	0.764753	4088	35.5478	36	28	43
1	6	22	93.5352	279.803	71	1.32937	0.658893	9.50789	0.922078	0.405818	7242	102	100	82	128
1	6	23	96.1847	458.115	157	1.38708	0.692999	14.1386	0.928994	0.747619	3965	25.1274	26	19	32
1	6	24	99	83	125	1.30866	0.645047	12.6157	0.919318	0.757516	4957	39.656	42	32	48
1	6	25	105.361	129.131	91	2.17195	0.887703	10.7641	0.919192	0.758333	3734	41.033	43	28	52
1	6	26	108.549	347.043	162	1.8889	0.848367	18.3619	0.905028	0.736364	4389	27.0926	28	21	34
1	6	27	119.701	107.444	97	1.81146	0.833817	11.1132	0.898148	0.621795	3817	39.3505	42	30	49
1	6	28	124.658	71.4658	234	2.15452	0.885761	17.2609	0.795918	0.65	8432	36.0342	37	28	45
1	6	29	129.865	443.19	237	2.14176	0.88431	17.3712	0.929412	0.593985	8025	34.0102	39	30	46

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1	6	30	132.384	470.016	250	1.71834	0.813219	17.8412	0.929368	0.657895	5098	20.392	21	16	25
1	6	31	124.67	378.226	115	1.36192	0.692339	12.1005	0.891473	0.69697	4161	36.1826	38	28	44
1	6	32	133.392	331.248	153	1.70893	0.810918	13.9573	0.910714	0.708333	4400	28.7582	29	22	35
1	6	33	129.878	361.081	99	1.51368	0.750701	11.2272	0.9	0.692308	4268	43.1111	45	34	51
1	6	34	135.862	376.505	196	1.68408	0.804616	15.7973	0.806584	0.640523	9241	67.148	49	37	55
1	6	35	136.281	415.62	171	1.60258	0.78143	14.7355	0.909574	0.678571	3972	23.2781	24	18	28
1	6	36	137.944	332	118	1.86552	0.844191	12.2573	0.914729	0.670455	4024	34.1017	36	26	42
1	6	37	143.395	40.8098	205	2.45832	0.913525	16.1559	0.923423	0.718783	4596	22.4195	24	17	28
1	6	38	136.271	497.308	107	2.61368	0.923925	11.672	0.89916	0.629412	4016	37.5327	38	28	47
1	6	39	141.448	248.271	181	3.38148	0.689966	15.1808	0.918762	0.709804	8458	64.7293	47	37	57
1	6	40	153.392	90.6022	181	1.20958	0.582591	15.1808	0.93299	0.760301	8163	45.0991	47	37	55
1	6	41	155.006	138.468	173	1.63704	0.791746	14.8415	0.910526	0.720833	7660	44.2775	45	35	53
1	6	42	154.19	371.076	105	1.57167	0.771469	11.5424	0.9375	0.777778	4529	43.1333	45	35	50
1	6	43	158.17	311.535	159	1.82167	0.835858	14.2283	0.913793	0.722727	4453	28.0063	29	21	35
1	6	44	163.866	82.5155	97	1.30584	0.643091	11.1132	0.92381	0.746154	4091	42.1753	45	35	50
1	6	45	163.836	193.575	73	1.19543	0.547937	9.64088	0.879518	0.663636	2333	31.9589	32	26	39
1	6	46	164.629	443.971	105	1.30539	0.642774	11.5424	0.905172	0.734266	4096	39.0095	40	30	48
1	6	47	169.383	77.1358	81	1.09464	0.410568	10.1554	0.89011	0.736264	3980	49.1358	50	40	60
1	6	48	171.28	161.194	93	1.48321	0.738516	10.8817	0.93	0.704515	3451	37.1075	39	30	43
1	6	49	171.794	405.46	126	1.15273	0.49743	12.666	0.933333	0.807692	3872	30.7302	32	24	37
1	6	50	171.641	492.672	128	1.87546	0.865987	12.7662	0.855105	0.627451	4145	32.3821	34	25	40
1	6	51	173.67	222.276	203	1.35599	0.675379	16.0769	0.931193	0.751852	8986	44.266	46	34.	53
1	6	52	178.902	433.735	102	1.81242	0.83101	11.3961	0.87931	0.60355	4289	42.019	43	31	54
1	6	53	185.608	66.8987	237	2.73651	0.931981	17.3712	0.792612	0.50441	8974	37.865	41	29	47
1	6	54	194.827	300.864	162	1.81442	0.834412	14.3619	0.936416	0.77512	4457	27.5123	28	21	34
1	6	55	196.489	333.525	282	1.85588	0.842417	18.9487	0.88125	0.582645	9826	34.844	37	28	42
1	6	56	193.333	198.188	69	1.37758	0.68779	9.37302	0.907895	0.784091	2189	31.7246	33	24	39
1	6	57	197.394	480.986	142	1.38735	0.693144	13.4462	0.910256	0.682692	4317	30.4014	31	24	37
1	6	58	198.895	14.0349	172	1.53588	0.758998	14.7986	0.919786	0.671875	8451	49.1337	51	40	60
1	6	59	203.48	182.922	103	1.19441	0.546851	11.4518	0.927928	0.780303	4291	41.6602	44	34	50
1	6	60	203.9	144.811	90	1.15049	0.494473	10.7047	0.909091	0.743802	4030	44.7778	47	35	54
1	6	61	208.575	113.591	127	1.13098	0.467075	12.7162	0.894366	0.697802	4249	33.4567	34	25	43
1	6	62	211.252	442.087	127	1.54916	0.763752	12.7162	0.907143	0.721591	4218	33.2126	35	26	40
1	6	63	216.444	54.7969	192	1.19242	0.5447	15.6353	0.923077	0.705882	8452	45.0625	46	35	57
1	6	64	217.935	201.412	148	1.21036	0.563371	13.7273	0.930818	0.704762	6994	47.2568	50	38	57
1	6	65	216.428	175.738	164	1.70375	0.809629	14.4503	0.921348	0.744689	5629	34.3237	36	28	40
1	6	66	218.676	394.858	204	2.15038	0.885288	16.1165	0.910714	0.83354	4637	22.7304	24	18	27
1	6	67	220.865	350.827	104	1.29582	0.635975	11.5073	0.928571	0.722222	4228	40.6538	42	31	49
1	6	68	229.603	236.64	138	1.42958	0.714626	13.159	0.877419	0.653846	3947	29.0221	30	22	36
1	6	69	228.632	467.226	106	1.30212	0.690294	11.6174	0.850356	0.688312	4010	37.8302	39	31	46
1	6	70	240.147	249.049	143	2.56519	0.920885	13.4935	0.89375	0.525735	4059	28.3846	29	21	37
1	6	71	236.88	341.892	83	1.41995	0.709952	10.28	0.882979	0.709402	4079	49.1446	50	38	61
1	6	72	244.499	88.3706	143	1.38306	0.690813	13.4935	0.916667	0.744792	4294	30.028	31	23	37
1	6	73	251.232	18.872	125	1.2051	0.558056	12.6157	0.905797	0.744048	4150	33.2	34	27	40
1	6	74	253.463	309.288	80	1.63102	0.789994	10.0925	0.909091	0.740741	3944	49.3	51	39	61
1	6	75	257.025	44.1698	159	1.56254	0.76839	14.2283	0.929825	0.80303	4305	27.0755	28	21	33
1	6	76	258.014	278.879	180	1.7642	0.823835	13.3512	0.915033	0.625	4146	29.6143	31	22	37
1	6	77	257.368	332.333	87	1.40256	0.701183	10.5248	0.887755	0.725	4350	50.4598	51	38	63
1	6	78	258.619	367.266	124	1.03654	0.263166	12.5651	0.911765	0.733728	4215	33.9918	36	26	42
1	6	79	269.735	297.283	113	1.35843	0.676824	11.9948	0.904	0.733766	3856	34.1239	35	26	42
1	6	80	273.347	97.7288	118	1.47984	0.737133	12.2573	0.880597	0.648352	3983	33.7542	35	26	42
1	6	81	271.644	129.856	118	1.90244	0.850706	12.2573	0.893939	0.670455	3988	33.7966	35	26	41
1	6	82	281.493	495.375	236	2.40868	0.903746	19.4134	0.902439	0.560606	18228	61.5811	61	46	74
1	6	83	288.973	400.997	347	2.92475	0.939733	21.6166	0.882212	0.50551	14136	38.5177	40	31	47
1	6	84	288.48	201.696	125	1.10917	0.432621	12.6157	0.932836	0.801282	4211	33.688	35	26	41
1	6	85	289.615	433.362	96	1.49853	0.744771	11.0558	0.90566	0.738462	4561	47.5104	50	37	56
1	6	86	293.636	475.985	46	1.19599	0.548538	9.167	0.90411	0.733333	7232	109.576	112	84	134
1	6	87	299.794	390.016	126	2.50588	0.916924	12.666	0.9	0.736842	4331	34.373	35	26	43
1	6	88	304.173	264.688	301	1.1756	0.525769	19.5766	0.847887	0.651515	18051	59.9701	61	47	74
1	6	89	301.379	149.345	87	1.32239	0.654335	10.5248	0.90625	0.725	3930	45.1724	46	33	57
1	6	90	306.688	432.152	112	1.39275	0.696038	11.9416	0.903226	0.717949	4766	42.7321	45	33	51
1	6	91	305.333	335.758	33	1.52246	0.754039	6.48204	0.916667	0.673469	5488	166.303	170	138	199
1	6	92	310.047	193.859	128	1.81317	0.831161	12.7662	0.914286	0.609524	4232	33.0625	35	26	39
1	6	93	310.465	48.1628	129	1.19976	0.552515	12.8159	0.889655	0.661538	4033	31.2636	33	24	37
1	6	94	319.984	165.903	124	1.29066	0.63221	12.5651	0.925373	0.733728	4261	34.3629	35	26	42
1	6	95	325.932	374.114	235	1.66	0.843179	17.2977	0.925197	0.658263	8846	37.6426	39	29	43
1	6	96	328.701	433.028	87	1.30457	0.612199	10.5248	0.915789	0.725	4236	48.6897	51	41	56
1	6	97	325.081	446.453	86	1.70754	0.810574	10.4642	0.934783	0.826923	4283	49.8023	51	42	59
1	6	98	331.918	24.2634	243	2.42412	0.910948	17.5897	0.815436	0.54	8682	36.3514	37	27	45
1	6	99	331.921	91.5444	112	1.38632	0.692587	11.9416	0.918033	0.666667	4149	37.0446	39	30	43
1	6	100	332.461	151.078	128	1.36318	0.679407	12.7662	0.907801	0.711111	7759	60.6172	62	49	74
1	6	101	335.986	222.69	142	1.72276	0.814285	13.4462	0.934211	0.788889	4647	32.7254	34	26	38
1	6	102	350.636	153.236	110	1.39529	0.697384	11.8345	0.901639	0.714286	4340	39.4545	40	30	47
1	6	103	354.773	470.773	128	1.72529	0.814469	12.7662	0.927536	0.711111	4253	33.2266	34	25	41
1	6	104	356.548	434.774	217	1.67429	0.80204	16.4221	0.923404	0.758741	9279	42.7604	44	34	53
1	6	105	351.339	292.398	118	1.22631	0.576819	12.2573	0.914729	0.702381	4199	35.5847	37	28	44

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1	6	106	359.018	312.36	114	1.78064	0.827412	12.0478	0.997638	0.626374	4181	36.6751	38	28	43
1	6	107	361.744	169.031	129	1.60135	0.781048	12.8159	0.908451	0.661538	7536	58.4186	60	46	71
1	6	108	370.42	126.786	112	1.12786	0.46247	11.9416	0.903226	0.117917	4218	37.6607	40	30	46
1	6	109	379.866	101.258	97	1.21577	0.568727	11.1132	0.898148	0.734848	4144	42.7216	44	34	51
1	6	110	382.727	404.684	187	1.71828	0.820259	15.4304	0.912195	0.611111	8630	46.1197	46	36	57
1	6	111	388.96	193.188	303	1.82054	0.835633	19.6416	0.907198	0.63125	11210	36.9967	39	27	43
1	6	112	387.155	337.22	174	1.75594	0.821995	14.8442	0.915789	0.725	8757	50.3216	52	39	61
1	6	113	384.468	261.441	136	1.64261	0.804233	13.159	0.894737	0.653846	6577	33.6548	35	27	41
1	6	114	401.428	302.432	438	1.51997	0.753098	22.9591	0.846109	0.513333	19684	47.5459	50	37	61
1	6	115	399.517	232.544	149	2.01919	0.872845	13.7736	0.925444	0.636752	5611	36.3154	38	29	44
1	6	116	411.693	273.039	384	1.50902	0.748901	22.1116	0.879374	0.601881	9995	26.0746	27	20	32
1	6	117	426.434	70.7862	159	1.6314	0.790105	14.2282	0.940828	0.80303	4597	28.9105	29	22	35
1	6	118	429	14	109	1.24326	0.594169	11.7806	0.931624	0.762238	3903	35.8073	37	30	42
1	6	119	438.508	178.255	132	1.38336	0.690966	12.9641	0.923077	0.733333	4152	31.4545	33	25	38
1	6	120	442.846	371.2	395	1.68433	0.80468	22.4261	0.951807	0.801217	10217	25.8658	28	20	31
1	6	121	435.648	108.209	91	1.47734	0.733551	10.7641	0.91	0.777778	3764	41.3626	43	33	51
1	6	122	439.969	245.441	131	1.74057	0.818488	12.9149	0.891156	0.727778	4284	32.7022	34	25	39
1	6	123	444.345	472.391	110	1.57612	0.755107	11.8345	0.92437	0.785714	4249	38.8091	40	29	47
1	6	124	447.904	87.8989	178	1.59428	0.775618	15.0515	0.92228	0.669173	4498	25.2697	27	20	30
1	6	125	450.265	488.34	113	1.37601	0.686912	11.9948	0.941667	0.807143	4363	38.6106	39	30	48
1	6	126	454.785	140.813	107	1.25762	0.606412	11.672	0.90678	0.788252	3888	36.3364	38	29	44
1	6	127	456.12	159.5	100	1.2749	0.620284	11.2838	0.925926	0.769231	3858	38.58	41	31	47
1	6	128	461.271	117.624	133	1.7004	0.808751	13.0131	0.875	0.59375	4162	31.2932	33	24	38
1	6	129	463.58	186.898	257	1.7078	0.810437	18.0893	0.911348	0.673894	9420	36.6537	38	28	46
1	6	130	472.762	297.525	101	1.12911	0.464356	11.3401	0.90991	0.765152	8080	80	79	63	96
1	6	131	477.607	138.476	145	1.23853	0.589392	13.5875	0.923567	0.739796	4309	29.7172	30	23	38
1	6	132	477.543	342.586	116	1.10392	0.423583	12.153	0.913386	0.74359	4237	36.5259	39	30	43
1	6	133	479.45	22.1651	109	1.42045	0.710199	11.7806	0.923729	0.778571	4270	39.1743	40	31	48
1	6	134	485.604	60.2073	164	2.07652	0.876405	14.4503	0.916201	0.780952	5044	30.7561	33	25	37
1	6	135	480.397	168.305	63	1.41513	0.707564	8.95623	0.851351	0.63	6763	107.349	109	85	134
1	6	136	490.58	251.707	178	1.55756	0.766678	14.8843	0.935484	0.74359	6842	39.3218	41	31	47
1	6	137	487.806	313.887	62	1.60841	0.783231	8.88487	0.898551	0.805195	3893	62.7903	65	51	75
1	6	138	496.029	331.5	208	2.09048	0.878165	16.2737	0.822134	0.521303	8882	42.7019	43	32	53
1	6	139	498.219	7.92708	96	1.10598	0.427089	11.0558	0.90566	0.727273	4030	41.9792	44	33	50
1	6	140	498.817	358.104	115	1.19899	0.551714	12.1005	0.942623	0.804196	4250	36.9565	38	29	44
1	6	141	501.108	502.892	93	1.00155	0.0555984	10.8817	0.920792	0.768595	3878	41.6989	42	32	50
1	7	1	13.1717	58.4565	99	1.42756	0.713666	11.2272	0.891892	0.707143	3940	39.798	40	32	48
1	7	2	22.2857	68.4388	98	1.39204	0.695662	11.1704	0.890909	0.7	3938	40.1837	42	32	47
1	7	3	24.3438	345.156	96	1.19415	0.54657	11.0558	0.923077	0.727273	4129	43.0104	44	34	53
1	7	4	31.2399	23.5351	271	1.67369	0.801881	18.5755	0.783237	0.654589	8489	31.3247	32	24	39
1	7	5	27.97	130.52	100	1.30865	0.645736	11.2838	0.925926	0.769231	4244	42.44	46	34	51
1	7	6	30.458	144.626	131	1.82018	0.835562	12.9149	0.903448	0.727778	5650	43.1908	44	34	52
1	7	7	30.0086	486.681	116	1.49932	0.745087	12.153	0.913386	0.686391	4109	35.4224	37	28	43
1	7	8	38.7195	155.928	221	1.71096	0.811418	16.7746	0.928571	0.701587	5177	23.4253	24	18	29
1	7	9	35.513	379.287	115	1.15651	0.502344	12.1005	0.905512	0.737179	4277	37.1913	38	29	46
1	7	10	35.7714	414.6	35	1.37755	0.687772	6.67558	0.897436	0.728167	5404	154.4	164	120	194
1	7	11	46.23	272.97	100	1.08978	0.397477	11.2838	0.917431	0.757576	4182	41.82	43	34	50
1	7	12	49.9759	76.6747	166	1.22086	0.583048	14.5381	0.917127	0.741071	8273	49.8373	51	40	59
1	7	13	49.7219	137.887	151	1.18031	0.737328	13.8658	0.920732	0.719048	5049	33.4371	35	26	40
1	7	14	53.4902	91.1242	153	1.67967	0.803464	13.9573	0.910714	0.772727	8574	56.0392	58	44	67
1	7	15	50.2083	30.3333	96	1.56263	0.768415	11.0558	0.897196	0.666667	3591	37.4063	40	30	45
1	7	16	66.7215	223.744	219	1.88308	0.847343	16.6985	0.939914	0.731039	4999	22.8265	24	18	28
1	7	17	68.2202	489.917	109	1.93494	0.8561	11.7806	0.872	0.605556	4065	37.2936	38	28	47
1	7	18	77.8481	261.287	308	1.46838	0.732245	11.7265	0.915254	0.72	4321	40.0093	42	33	49
1	7	19	79.8741	132.719	135	1.65219	0.796019	13.1106	0.9	0.642857	4592	34.0148	36	26	42
1	7	20	78.1333	311.013	75	1.25902	0.60757	9.77205	0.914634	0.757576	3898	51.9733	53	42	62
1	7	21	79.1449	472.848	138	1.26926	0.61585	13.2555	0.926174	0.766667	4437	32.1522	33	25	39
1	7	22	86.9679	277.058	156	2.24156	0.894972	14.0935	0.928571	0.825397	4699	30.1218	31	23	37
1	7	23	85.6039	368.268	153	1.70677	0.810381	13.9573	0.916168	0.732057	4526	29.5817	30	23	37
1	7	24	91.7971	43.0638	345	1.77297	0.825758	20.9587	0.8625	0.709877	13087	37.9333	38	30	46
1	7	25	88.3014	321.219	73	1.28553	0.628198	9.64088	0.935897	0.737374	3957	54.2055	55	43	65
1	7	26	95.0667	101.005	195	1.76841	0.824763	15.757	0.842029	0.77381	9668	49.5795	52	41	60
1	7	27	94.0161	187.977	174	1.5115	0.749861	14.8843	0.95082	0.78733	4673	26.8563	28	21	33
1	7	28	95.9318	388	132	1.57466	0.772463	12.9641	0.891892	0.628571	4467	33.8409	35	26	41
1	7	29	96.8947	297.728	114	1.38869	0.693466	12.0478	0.897638	0.74026	4073	35.7807	36	28	44
1	7	30	104.784	86.024	167	2.0048	0.866715	14.5819	0.922652	0.722944	9204	55.1138	57	43	66
1	7	31	100.91	242.72	100	1.33022	0.659442	11.2838	0.917431	0.769231	4252	42.52	43	33	51
1	7	32	105.849	492.497	159	1.88554	0.847777	14.2283	0.903409	0.639487	4668	29.3585	30	23	36
1	7	33	108.051	161.037	136	1.77383	0.825944	13.159	0.918919	0.8	4218	31.0147	31	23	38
1	7	34	114.5	219.5	148	1.5001	0.745397	13.7273	0.91358	0.660714	4563	30.8311	32	23	39
1	7	35	112.944	348.88	125	1.12674	0.46016	12.6157	0.912409	0.744048	4232	33.856	34	27	41
1	7	36	122.696	389.301	359	2.25956	0.896739	21.3797	0.8975	0.543939	21578	60.1058	60	43	76
1	7	37	118.715	407.016	123	1.96567	0.860924	12.5143	0.911111	0.585714	4530	36.8293	38	29	45
1	7	38	125.698	131.671	149	1.82258	0.836038	13.7736	0.908537	0.712919	4381	29.4698	30	23	36
1	7	39	127.937	335.779	190	1.73451	0.817074	15.5536	0.892019	0.695971	4777	25.1421	26	20	31
1	7	40	129.657	289.657	105	1.29891	0.638191	11.5624	0.913043	0.729167	3997	38.0667	40	29	47

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1	7	41	132.831	416.085	318	1.57941	0.77403	12.2573	0.929134	0.7375	4370	37.0339	39	29	44
1	7	42	141.089	179.444	192	1.86987	0.844981	15.6353	0.91866	0.627451	4735	24.7656	25	19	33
1	7	43	146.275	477.265	290	1.81175	0.833875	19.4788	0.823204	0.525573	9510	31.9128	33	25	39
1	7	44	145.752	312.468	141	1.3543	0.499474	13.3988	0.915584	0.723077	4314	30.5957	32	24	37
1	7	45	148.67	36.2611	203	1.35341	0.674085	26.0769	0.944188	0.763158	9182	45.2315	47	35	55
1	7	46	151.921	219.534	189	1.68864	0.906053	15.5128	0.935644	0.75	4903	25.9418	26	20	32
1	7	47	150.319	93.6746	169	1.91994	0.853632	18.6489	0.923497	0.470635	4649	27.5089	29	21	34
1	7	48	155.872	265.497	187	2.03842	0.872425	15.4304	0.923192	0.836054	7571	40.4866	42	31	49
1	7	49	155.578	139.748	135	1.54598	0.76404	13.1106	0.912182	0.767045	4292	31.7926	33	25	39
1	7	50	164.323	388.369	198	2.46046	0.913683	15.0777	0.92093	0.6875	5235	26.4394	27	20	32
1	7	51	177.089	501.03	336	3.04299	0.944461	20.6835	0.835823	0.738462	9838	29.2798	31	21	37
1	7	52	175.35	180.975	160	1.74376	0.819225	14.273	0.91954	0.683761	4476	27.975	29	22	34
1	7	53	182.463	312.893	177	2.06497	0.874919	15.0121	0.907692	0.578431	6096	34.4407	36	27	41
1	7	54	182.062	31.9482	193	1.13599	0.476432	35.6759	0.881279	0.709559	8458	43.829	44	34	53
1	7	55	183.867	145.944	163	1.68217	0.804117	13.4935	0.899371	0.647059	4328	30.2657	31	24	37
1	7	56	183.435	436.351	131	1.32047	0.653061	12.9149	0.935714	0.779762	4343	33.1527	34	26	39
1	7	57	191.742	254.049	308	3.6833	0.804152	22.2265	0.864183	0.666667	19335	49.8325	52	39	60
1	7	58	187.664	466.48	152	1.63687	0.791721	13.9116	0.921212	0.745098	4421	29.0655	30	22	36
1	7	59	191.774	354.361	208	2.11997	0.881757	16.2737	0.920354	0.630303	8950	43.0288	44	33	52
1	7	60	199.537	34.8299	147	2.09237	0.8784	13.6409	0.924528	0.6125	4256	28.9526	30	23	34
1	7	61	199.765	391.686	226	1.16339	0.511034	16.9633	0.933884	0.738562	8912	39.6384	42	30	49
1	7	62	202.694	117.496	121	1.8066	0.832832	12.4122	0.883212	0.617059	4298	35.5207	37	27	44
1	7	63	223.218	472.565	156	1.52289	0.754197	14.0935	0.904977	0.696429	4265	27.3397	28	21	33
1	7	64	223.645	75.8455	110	1.67258	0.601587	11.8365	0.92437	0.733333	4320	57.4545	61	46	69
1	7	65	225.35	212.617	206	1.72215	0.814138	16.1953	0.923767	0.754579	4394	21.3301	22	16	27
1	7	66	230.35	311.387	160	2.02402	0.869424	14.273	0.930233	0.8	7553	47.2062	50	37	57
1	7	67	229.214	47.3761	117	1.78822	0.828238	12.2053	0.9	0.664773	6538	55.8803	59	44	67
1	7	68	230.252	426.611	143	1.50452	0.747143	13.4915	0.916667	0.680952	4484	31.3566	32	23	39
1	7	69	233.37	135.318	127	1.19803	0.550763	12.7162	0.92029	0.755932	4349	34.2441	35	26	42
1	7	70	239.567	368.239	180	1.3291	0.658072	15.1388	0.923077	0.703125	9241	51.3309	53	40	64
1	7	71	245.931	256.523	243	1.81026	0.833575	17.5897	0.94186	0.778846	5636	23.1934	24	18	28
1	7	72	246.938	393.8	130	1.46474	0.799475	12.8455	0.921986	0.666667	4446	34.2	35	26	43
1	7	73	254.428	478.726	317	1.58648	0.778396	20.0302	0.878116	0.400379	14100	44.4795	44	32	57
1	7	74	251.756	330.701	127	1.50086	0.745619	12.7162	0.913669	0.647959	4629	36.4688	37	29	45
1	7	75	260.117	438.662	145	2.16787	0.887254	13.5875	0.895062	0.635965	4313	29.7449	30	24	37
1	7	76	272.667	226.453	285	2.07848	0.878654	19.0492	0.92233	0.633333	7234	25.593	26	20	31
1	7	77	271.121	89.2369	149	1.58153	0.774724	13.7736	0.91441	0.678208	4394	29.5034	30	22	37
1	7	78	271.065	418.276	123	1.4177	0.70384	12.5143	0.938931	0.727811	4288	34.8618	37	28	41
1	7	79	275.814	457.496	129	1.19877	0.551482	12.8159	0.902038	0.706791	6334	33.5969	35	26	41
1	7	80	281.035	107.674	166	1.36406	0.680815	13.5406	0.942178	0.8	4390	30.4863	32	23	36
1	7	81	283.372	378.194	180	1.37189	0.684597	15.1388	0.932642	0.75	8550	47.5444	49	37	59
1	7	82	291.019	305.957	208	1.55843	0.766901	16.2737	0.924444	0.742857	9450	45.4327	48	35	56
1	7	83	291.444	425.593	135	1.92398	0.851318	13.1106	0.944056	0.833333	4238	31.3926	32	25	38
1	7	84	302.061	495.628	160	1.18433	0.538012	14.4503	0.906077	0.489076	5496	33.5122	35	25	42
1	7	85	326.196	440.576	715	2.71041	0.929881	30.1723	0.657169	0.381956	20112	28.1287	29	21	35
1	7	86	318.028	379.6	180	1.80836	0.833189	15.1388	0.909091	0.625	8010	44.5444	46	34	55
1	7	87	319.139	480.525	122	1.19159	0.547796	12.4634	0.903708	0.72619	4028	33.0144	35	27	39
1	7	88	327.388	391.964	165	1.73598	0.817419	14.4943	0.901639	0.651762	4708	28.5212	30	23	34
1	7	89	338.806	170.942	191	2.02727	0.868874	15.5945	0.896714	0.606349	8694	45.5183	48	37	54
1	7	90	344.676	379.211	71	1.67896	0.803276	9.50789	0.910256	0.739583	4011	56.493	57	44	69
1	7	91	353.818	364.282	213	2.08015	0.876904	16.4482	0.918103	0.531667	8798	41.3052	42	32	50
1	7	92	362.19	91.9241	158	1.5969	0.779453	14.1835	0.913295	0.663866	4870	30.8226	32	24	38
1	7	93	362.172	330.44	87	1.04982	0.306404	10.5244	0.925788	0.723008	3702	42.5537	44	35	52
1	7	94	361.328	388.148	61	1.41465	0.707325	8.81292	0.884058	0.7625	3820	62.623	64	51	76
1	7	95	377.571	322.073	259	1.66998	0.800892	18.1595	0.863333	0.6175	13664	52.7568	51	39	67
1	7	96	380.455	482.961	231	2.67979	0.927743	17.1499	0.916667	0.712963	5820	25.1948	26	19	31
1	7	97	383.223	355.843	121	1.30452	0.642149	12.4122	0.916667	0.733333	4088	33.7851	34	27	41
1	7	98	398.118	460.118	341	2.14008	0.884113	11.3175	0.909605	0.557093	5244	32.5714	34	26	39
1	7	99	395.224	337.138	76	1.55327	0.765132	9.83898	0.908762	0.730769	3878	51.0263	53	40	62
1	7	100	400.148	234.995	203	1.43778	0.791956	16.0769	0.922727	0.667763	8751	43.1084	45	32	55
1	7	101	401.29	274.575	183	1.85013	0.861359	15.6759	0.923465	0.630719	6950	38.0104	37	27	44
1	7	102	405.227	381.542	162	1.23011	0.582352	13.4462	0.922078	0.728205	4346	30.6056	32	23	37
1	7	103	416.389	360.643	126	1.31233	0.647598	12.666	0.913043	0.75	4165	33.0556	34	26	40
1	7	104	423.382	433.085	436	1.8334	0.838154	23.5612	0.872	0.619318	18633	42.7342	44	33	53
1	7	105	429.247	58.6533	150	1.28378	0.627084	13.8159	0.931677	0.78125	4218	28.12	29	22	34
1	7	106	437.237	209.237	38	1.71276	0.813959	6.9558	0.826087	0.59375	3012	73.2632	80	61	100
1	7	107	442.343	200.857	35	1.44058	0.719817	6.67556	0.875	0.729167	2872	82.0571	82	60	100
1	7	108	460.931	222.448	174	1.75735	0.82231	14.8843	0.935484	0.750909	5908	33.954	35	27	40
1	7	109	463.61	277.59	105	1.78648	0.828655	11.5624	0.897436	0.625	3901	37.1524	38	30	44
1	7	110	466.514	321.648	122	1.43784	0.708913	12.4634	0.917293	0.72619	4585	37.582	39	30	45
1	7	111	473.447	121.924	119	1.70559	0.810088	12.3093	0.888613	0.610256	3984	33.479	35	26	40
1	7	112	482.814	335.798	114	1.13186	0.469389	12.0478	0.934426	0.797203	4228	37.0877	39	29	44
1	7	113	486.5	287.192	108	1.15544	0.500955	11.5073	0.886552	0.727273	3993	37.4327	39	29	45
1	7	114	497.774	121.27	115	1.64336	0.728103	12.1005	0.881473	0.69887	3920	34.087	35	27	41
1	7	115	499.638	314.244	127	1.47025	0.735067	12.7162	0.913669	0.705554	4434	34.9291	37	28	41
1	7	116	502.024	134.756	156	1.15883	0.505312	14.0935	0.939759	0.8	4744	30.4103	32	23	37

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1	8	1	11.1154	387.507	100	1.42586	0.712837	11.5073	0.904348	0.675325	3955	38.0288	39	31	46
1	8	2	9.80303	428.712	66	1.2069	0.559891	9.167	0.88	0.733333	3059	46.3485	48	40	53
1	8	3	11.6364	443.818	77	1.17652	0.528646	9.90149	0.927711	0.77	2990	39.8312	40	34	44
1	8	4	14.4143	456.586	70	1.03328	0.251744	9.4407	0.886076	0.7	3970	56.7143	58	46	68
1	8	5	18.3947	45.1316	114	1.263	0.610824	12.0470	0.912	0.730769	3892	34.1404	35	27	41
1	8	6	21.18	172.58	100	1.52012	0.753154	11.2038	0.884956	0.714286	3840	38.4	39	31	47
1	8	7	25.1143	499.143	140	1.32652	0.758554	13.3512	0.903226	0.673077	4072	29.0857	30	22	36
1	8	8	25.8148	433.305	189	1.85563	0.859345	15.5126	0.836283	0.63	12608	66.709	69	51	84
1	8	9	25.8947	150.814	114	1.27031	0.616885	12.0470	0.919355	0.730769	4004	35.1228	37	28	43
1	8	10	27.688	113.544	125	1.44333	0.721089	12.6157	0.905797	0.694444	7138	57.104	60	44	69
1	8	11	27.9023	349.684	133	-1.23436	0.58642	13.0131	0.917241	0.738889	4208	31.6391	32	25	39
1	8	12	28.6991	66.8407	113	1.33116	0.660045	11.9948	0.91129	0.724359	4075	36.0619	37	29	44
1	8	13	30.7857	380.439	196	1.80157	0.831803	15.7973	0.903226	0.606811	5557	28.352	30	22	35
1	8	14	36.7762	311.455	143	1.07665	0.374987	13.4935	0.922581	0.785714	4063	28.4126	29	23	34
1	8	15	49.0118	37.619	252	1.38306	0.694066	17.9125	0.82623	0.572727	11427	45.3452	45	34	58
1	8	16	45.7623	123.623	122	1.10508	0.425606	12.4634	0.917293	0.782051	4642	38.2131	39	30	46
1	8	17	46.7836	203.731	134	1.60438	0.781987	13.0619	0.937043	0.683673	4152	30.9851	32	25	37
1	8	18	50.717	68.5566	212	1.49816	0.744624	16.4294	0.913749	0.69281	8804	41.5283	42	32	52
1	8	19	53.3043	461.739	92	1.44718	0.722855	10.823	0.910891	0.69697	3641	39.5761	39	31	49
1	8	20	66.0732	162.182	478	1.03578	0.260563	24.67	0.910476	0.735385	14880	31.1297	32	24	38
1	8	21	64.6333	121.408	120	1.21481	0.567792	12.3608	0.895522	0.710059	4070	33.9167	35	27	41
1	8	22	68.3801	770.181	221	1.5985	0.780155	16.7744	0.936441	0.736667	7891	35.7059	37	28	43
1	8	23	64.7756	339.358	156	1.54808	0.763371	14.0935	0.923077	0.787879	4298	27.5513	28	21	34
1	8	24	66.2913	50.0764	144	2.23108	0.893926	13.5406	0.947368	0.8	5206	36.1528	37	28	44
1	8	25	67.2043	473	93	1.33433	0.662074	10.8813	0.902913	0.65035	3739	40.2043	41	33	49
1	8	26	72.4838	297.915	117	1.44204	0.720491	12.2053	0.906977	0.709091	3961	33.8547	34	26	41
1	8	27	72.387	401.874	189	2.01167	0.867693	15.5126	0.917476	0.642857	5541	29.3175	30	23	36
1	8	28	76.8716	134.731	134	1.70364	0.809651	13.0619	0.911565	0.697917	4157	30.9851	33	24	37
1	8	29	74.6636	109.168	107	1.23867	0.590113	11.672	0.90678	0.748252	4122	38.5234	39	30	47
1	8	30	85.7585	188.102	147	2.37263	0.906881	13.6809	0.90184	0.668182	4231	28.7823	29	23	34
1	8	31	84.292	74.7611	113	1.16561	0.513778	11.9948	0.904	0.724359	4168	36.885	38	29	44
1	8	32	95.8089	208.8	403	1.49429	0.743068	22.6521	0.846639	0.648953	14872	36.9032	37	28	45
1	8	33	90.5596	140.101	109	1.57451	0.772416	11.7806	0.893443	0.64881	4053	37.1835	39	30	45
1	8	34	98.5458	463.579	273	1.91669	0.853109	18.6439	0.877814	0.538684	13953	51.1172	52	38	64
1	8	35	99.1137	247.933	299	1.67483	0.788176	19.5115	0.937304	0.684211	9570	32.0067	33	25	39
1	8	36	98.2744	37.556	277	1.98601	0.842255	18.78	0.882168	0.502722	7730	27.9061	29	21	34
1	8	37	98.7634	166.452	93	1.61096	0.801153	10.8817	0.885714	0.65035	3856	41.4624	42	32	50
1	8	38	108.512	493.148	205	2.13682	0.883736	16.1559	0.911111	0.741806	4628	22.5756	23	17	28
1	8	39	112.24	441.38	150	1.6123	0.784417	13.8198	0.931677	0.757576	7793	51.9533	53	40	64
1	8	40	111.812	222.763	117	1.95283	0.85894	12.2053	0.906977	0.744706	4458	38.1026	39	31	45
1	8	41	113.866	344.431	149	1.39604	0.690828	13.7736	0.91611	0.716346	4233	28.4094	30	21	34
1	8	42	120.83	431.358	106	1.31794	0.65137	11.4174	0.923825	0.815385	3923	37.0094	37	29	45
1	8	43	129.929	192.142	84	1.15603	0.50172	10.3418	0.903226	0.694215	2693	32.0595	33	25	38
1	8	44	137.19	454.365	252	1.89597	0.843596	17.9125	0.854237	0.672	9387	37.25	38	28	44
1	8	45	134.843	128.374	305	2.06323	0.874693	19.7043	0.918675	0.680804	8280	27.1475	28	21	34
1	8	46	141.141	215.84	318	1.50812	0.748552	20.1535	0.932749	0.693478	11477	35.9781	36	28	43
1	8	47	137.059	242.738	119	1.33313	0.661381	12.3092	0.915385	0.772727	3584	30.1176	31	24	36
1	8	48	145.367	17.8986	207	1.77509	0.82639	16.2345	0.924107	0.704082	8582	41.4589	44	32	50
1	8	49	153.778	413.183	115	1.65244	0.796105	12.1005	0.927419	0.680473	4040	35.1304	37	28	42
1	8	50	159.463	278.456	136	1.08378	0.341056	13.159	0.937931	0.747253	4230	31.1029	33	25	37
1	8	51	159.706	91.2327	245	2.00944	0.867377	17.6619	0.924528	0.753846	8913	36.3796	37	28	44
1	8	52	159.59	393.024	83	1.4086	0.70428	10.28	0.912088	0.691667	3838	46.241	47	37	57
1	8	53	164.983	500.36	175	1.69183	0.806417	14.9271	0.91623	0.643382	4480	25.6	26	20	31
1	8	54	170.853	257.131	375	1.34636	0.669575	21.851	0.910194	0.708885	14567	38.792	39	31	48
1	8	55	177.162	54.2575	167	1.52019	0.753181	14.5819	0.912568	0.701681	5095	30.509	32	24	36
1	8	56	178.6	307.358	95	1.20724	0.560236	10.9981	0.896226	0.719697	3826	40.2737	40	32	49
1	8	57	182.152	435.777	224	2.7007	0.928922	16.888	0.858238	0.518519	9007	40.2098	40	31	51
1	8	58	179.327	459.119	101	1.22641	0.578918	11.3401	0.926606	0.765152	4011	39.7129	41	33	48
1	8	59	183.359	190.781	128	1.23747	0.589043	12.7662	0.948148	0.820513	2772	21.6563	22	17	26
1	8	60	185.464	303.145	110	1.86455	0.844013	11.8345	0.887097	0.604396	4156	37.7818	39	29	47
1	8	61	187.036	138.323	222	1.63285	0.790527	16.8503	0.940928	0.724026	8737	39.3587	39	30	50
1	8	62	194.681	214.072	138	2.23437	0.894257	13.2555	0.907895	0.746667	4036	29.2464	30	22	36
1	8	63	199.681	455.08	113	1.71204	0.811682	11.9948	0.91129	0.684848	4036	35.7168	36	27	44
1	8	64	207.119	282.204	226	1.93002	0.855302	16.9633	0.896825	0.724359	7750	34.3097	36	27	41
1	8	65	203.444	35.0171	117	1.26158	0.409649	12.2053	0.92126	0.75974	3975	33.9744	35	26	42
1	8	66	206.23	259.208	178	1.36462	0.680437	15.0545	0.92228	0.706349	6679	37.5225	39	30	46
1	8	67	210.406	35.8125	128	1.45902	0.728174	12.7662	0.901408	0.711111	4017	31.3828	32	24	39
1	8	68	220.384	93.4399	461	1.13472	0.472599	24.2273	0.931313	0.770903	18442	40.0043	42	30	50
1	8	69	225.178	211.767	163	1.11492	0.44218	14.4062	0.926136	0.724444	4374	26.8344	28	21	33
1	8	70	230.979	235.904	146	1.31912	0.65216	13.6343	0.918239	0.695238	4134	28.3151	29	22	35
1	8	71	234.633	495.272	346	2.33131	0.903331	20.9891	0.800926	0.518741	10802	31.2197	33	23	39
1	8	72	235.502	132.659	255	1.7484	0.820288	18.0188	0.897887	0.643939	8918	34.9725	36	27	42
1	8	73	231.124	391.719	89	1.45186	0.724978	10.6451	0.89899	0.760684	4314	48.4719	47	38	59
1	8	74	242.741	156.606	251	2.36019	0.896197	17.8769	0.820261	0.603365	9132	36.3825	38	28	45
1	8	75	239.34	389.296	162	1.7497	0.820584	14.3619	0.89011	0.692308	4184	25.8272	26	20	31
1	8	76	238.314	24.8551	138	1.77073	0.825269	13.2555	0.92	0.657143	4080	29.5652	30	23	36

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1	8	77	241.36	45.3128	211	1.49785	0.808148	14.3907	0.914188	0.753571	8135	38.5545	40	30	47
1	8	78	241.66	308.241	191	1.60919	0.783169	13.5945	0.927184	0.773279	4521	23.6702	25	18	29
1	8	79	249.134	425.698	149	1.43414	0.780303	13.7738	0.93125	0.718316	4359	29.255	30	22	36
1	8	80	248.37	370.24	72	1.24745	0.597815	9.64088	0.890244	0.675926	3060	41.3178	43	35	51
1	8	81	248.378	398.218	154	2.20046	0.890793	14.0935	0.852459	0.6	8763	42.5833	59	47	80
1	8	82	259.588	266.39	182	1.84198	0.838801	13.2227	0.896552	0.594771	4383	23.333	25	19	29
1	8	83	272.398	203.522	251	2.33573	0.903714	17.8749	0.831126	0.518595	5000	35.0566	37	26	46
1	8	84	273.527	441.305	131	2.15733	0.886078	12.9149	0.89726	0.661616	4492	34.2901	34	26	42
1	8	85	273.769	485.615	143	1.38607	0.692189	13.4935	0.924571	0.744792	4198	29.3427	31	23	35
1	8	86	274.033	162.951	248	1.50594	0.7437	18.4128	0.930594	0.708995	8918	33.2761	35	26	40
1	8	87	278.313	457.261	150	1.60585	0.782443	13.8198	0.898204	0.694444	4322	24.8133	29	23	36
1	8	88	282.808	48.0113	177	1.13131	0.467421	15.0131	0.941489	0.784667	4236	23.9322	25	20	28
1	8	89	292.118	84	170	1.75669	0.822189	14.7123	0.918918	0.772727	4143	24.3706	26	19	29
1	8	90	293.917	412.439	332	1.32413	0.655478	17.9441	0.914667	0.723333	4542	49.5606	50	39	60
1	8	91	300.819	214.943	105	1.43507	0.717238	11.5424	0.905172	0.673077	3901	31.1524	39	30	45
1	8	92	301.375	345.235	136	1.35237	0.673321	13.159	0.931507	0.747253	7767	57.1103	59	46	69
1	8	93	305.395	44.2727	244	1.97196	0.861881	18.334	0.778781	0.597285	8734	33.0833	34	26	40
1	8	94	308.838	83.3276	116	1.25302	0.602565	12.153	0.899225	0.637363	6893	59.4224	61	47	73
1	8	95	311.872	109.156	179	1.46436	0.73138	15.0987	0.927481	0.726496	6657	37.1699	38	28	46
1	8	96	318.228	247.72	272	3.32353	0.934473	18.6097	0.874598	0.618182	9264	34.0588	35	26	42
1	8	97	320.663	478.596	312	1.59325	0.778498	19.9311	0.891429	0.615048	18606	59.6346	62	47	72
1	8	98	323.678	188.77	296	1.77625	0.824468	19.9134	0.87578	0.770833	9457	31.9493	33	24	38
1	8	99	328.286	156.173	335	1.62631	0.788615	20.6527	0.656777	0.744444	11483	43.2328	44	33	53
1	8	100	324.84	416.43	244	2.40222	0.909235	17.6258	0.893773	0.625641	9558	39.1721	41	30	47
1	8	101	329.622	216.832	118	1.49567	0.807594	12.3092	0.901515	0.653846	4050	34.0336	35	27	41
1	8	102	332.686	380.285	102	1.34389	0.668061	11.3981	0.910714	0.728571	3971	38.9314	40	30	47
1	8	103	340.491	119.404	306	1.12248	0.454237	11.4174	0.921739	0.736111	4013	37.8585	39	30	44
1	8	104	348.5	136.445	310	1.50007	0.745385	11.8345	0.901639	0.654762	4017	36.5182	38	28	44
1	8	105	347.429	346.747	91	1.93171	0.844821	10.7441	0.919192	0.631944	3658	40.1978	41	31	50
1	8	106	348.818	236.455	121	1.14875	0.492143	12.4122	0.923664	0.775641	4267	35.2314	37	27	43
1	8	107	352.032	288.525	158	1.65609	0.797133	14.1835	0.904016	0.692982	4752	30.0759	32	23	37
1	8	108	352.05	489.95	119	1.59372	0.778641	12.3092	0.925688	0.701142	4086	34.3361	35	27	41
1	8	109	352	395.853	75	1.84593	0.840551	8.77205	0.882353	0.441024	2332	47.0933	49	36	60
1	8	110	354.868	111.082	182	1.68499	0.804953	15.2227	0.928571	0.736842	7724	42.4505	44	33	51
1	8	111	360.267	337.283	180	1.12392	0.454455	15.1388	0.937835	0.75	8844	49.1333	52	39	59
1	8	112	360.8	294.428	105	1.37302	0.683237	11.5824	0.9375	0.807692	3915	31.2857	37	29	45
1	8	113	360.821	184.388	116	1.47774	0.734252	11.153	0.885496	0.70303	3985	34.3534	36	27	41
1	8	114	368.133	45.8433	180	1.3597	0.676984	15.1388	0.918367	0.666667	4291	23.8389	24	18	29
1	8	115	362.3	432.778	90	1.68708	0.805303	10.7047	0.909091	0.75	3719	41.3111	42	32	52
1	8	116	365.321	494.077	104	1.52379	0.754535	11.5073	0.912201	0.693333	3903	37.5288	38	31	44
1	8	117	366.154	314.398	91	1.31092	0.646601	10.7641	0.91	0.758333	3794	41.6923	42	33	51
1	8	118	372.23	175.807	113	1.36563	0.68102	12.3092	0.901515	0.708333	4159	34.0496	36	27	43
1	8	119	373.35	213.65	143	1.0509	0.30746	13.4935	0.916667	0.729592	4415	30.8741	33	24	38
1	8	120	374.943	76.1478	230	2.12981	0.882519	17.1127	0.855019	0.613333	8527	37.0739	38	29	46
1	8	121	379.519	247.039	131	1.34195	0.666857	12.9149	0.922535	0.71978	4452	33.9847	36	28	40
1	8	122	383.722	277	115	1.19250	0.544875	12.1005	0.927419	0.804196	4275	34.3043	38	30	43
1	8	123	389.102	93.1437	167	1.2844	0.629051	14.5019	0.932961	0.755650	8366	50.0958	52	39	60
1	8	124	394.136	163.603	184	1.22361	0.574201	15.3061	0.938776	0.773103	8747	47.538	50	37	57
1	8	125	403.325	17.596	302	2.38349	0.907731	18.6093	0.883041	0.719048	17263	57.1755	58	44	70
1	8	126	398.503	250.074	312	2.69457	0.928584	19.9311	0.850136	0.484472	9922	31.8013	33	25	39
1	8	127	398.388	394.443	93	1.43839	0.719074	10.8817	0.894231	0.715385	3834	41.4109	42	32	51
1	8	128	408.33	139.144	227	2.83374	0.935644	17.0007	0.885719	0.693478	4413	19.4105	19	15	25
1	8	129	417.063	122.789	175	1.49851	0.744744	14.9271	0.935879	0.747863	4396	25.12	26	20	31
1	8	130	419.559	405.314	102	1.13504	0.473065	11.3961	0.810714	0.708333	4035	39.5588	41	30	48
1	8	131	445.338	198.922	234	1.84451	0.840283	17.2609	0.917368	0.782609	9125	38.9957	41	31	47
1	8	132	444.946	88.2473	186	1.58902	0.777149	15.389	0.925373	0.775	8002	43.0215	46	34	51
1	8	133	447.058	270.362	127	1.23599	0.587712	12.7162	0.927007	0.751479	3960	31.1811	33	25	38
1	8	134	443.276	30.0783	76	1.12741	0.461785	9.83698	0.873563	0.690909	3362	44.2368	46	36	55
1	8	135	451.223	104.749	175	1.62912	0.78944	14.9271	0.925926	0.767544	7897	45.1257	47	36	54
1	8	136	451.809	422.008	110	1.33502	0.649941	11.8345	0.916667	0.705128	4025	36.5909	38	29	45
1	8	137	459.594	316.903	165	1.57515	0.772628	14.4943	0.873016	0.705128	5011	30.5515	31	24	37
1	8	138	461.477	119.946	111	1.09167	0.448851	11.8802	0.880952	0.632941	2516	31.6757	33	24	39
1	8	139	462	394	51	1.38033	0.689309	9.05824	0.910714	0.809524	7047	138.176	140	111	169
1	8	140	489.372	225.858	401	2.93477	0.940157	22.5958	0.798805	0.541161	20652	51.5012	51	39	64
1	8	141	475.079	114.763	76	1.77782	0.824805	9.83698	0.873563	0.478571	3058	40.2368	41	31	49
1	8	142	479.314	244.428	105	1.33337	0.66144	11.5624	0.897436	0.75	3522	33.5429	35	27	40
1	8	143	482.952	495.062	146	1.66438	0.799924	13.6343	0.906832	0.737374	5039	34.5137	35	27	42
1	8	144	487.34	149.102	197	1.83365	0.836202	15.8376	0.907834	0.643791	8402	42.6497	44	33	52
1	8	145	493.055	44.1978	91	1.37478	0.688216	10.7641	0.875	0.7	2990	32.8571	33	25	40
1	8	146	501.221	100.897	68	1.22476	0.577363	9.30485	0.883117	0.686869	3341	49.1324	51	38	59
1	9	1	22.1198	487.728	134	1.82898	0.837234	13.0613	0.924130	0.788235	4145	30.9328	32	24	37
1	9	2	14.3464	59.3893	131	1.71457	0.812301	12.9149	0.929078	0.770588	4166	31.8015	33	25	39
1	9	3	17.4405	303.758	162	1.48906	0.732548	14.3018	0.910112	0.680472	7203	44.463	44	35	54
1	9	4	14.3913	179.217	23	1.23548	0.58724	5.41152	0.844615	0.766667	2354	102.348	106	85	116
1	9	5	15.2593	149.593	27	1.0586	0.320094	5.86323	0.870968	0.75	2416	96.8889	97	76	123
1	9	6	24.0507	187.71	138	1.29486	0.635347	13.2555	0.926174	0.766667	5429	39.3406	41	32	48

1	9	7	21.2188	364.125	64	1.29361	0.631372	9.02703	0.874712	0.727273	3642	56.9063	57	44	68
1	9	8	24.0233	453.966	329	1.02666	0.837231	12.8159	0.921429	0.681538	3872	30.0155	30	23	37
1	9	9	35.6128	114.293	140	1.45761	0.72755	13.3512	0.909091	0.729167	4313	30.8071	31	23	36
1	9	10	39.8272	251.376	206	1.6479	0.75483	14.1953	0.919643	0.677632	9034	43.7573	45	35	53
1	9	11	38.5562	329.443	378	1.06005	0.331795	15.0545	0.927083	0.791111	9196	51.6429	52	38	64
1	9	12	39.3667	41.52	350	1.17673	0.527089	13.8198	0.920245	0.765306	4703	31.3333	33	25	38
1	9	13	38.3365	362.388	85	1.17648	0.527268	30.4031	0.894737	0.708333	3980	46.8235	49	39	57
1	9	14	45.223	24.1727	339	1.40448	0.703172	13.3034	0.958621	0.862424	4279	30.7842	32	25	36
1	9	15	57.3316	101.368	187	1.77165	0.825428	15.4304	0.912195	0.667857	4410	23.5829	24	18	29
1	9	16	55.8906	62.5274	146	1.61579	0.785475	13.6313	0.924051	0.693238	4298	29.4386	30	22	36
1	9	17	55.5476	126.333	126	1.31697	0.650118	12.666	0.9	0.692308	4098	32.5238	33	25	40
1	9	18	65.4198	170.968	324	2.47752	0.918923	20.3108	0.79803	0.482143	16200	50	52	39	61
1	9	19	61.8615	292.138	395	1.65939	0.798019	15.757	0.919811	0.714286	8025	46.2921	48	35	57
1	9	20	63.3444	214.022	380	1.7726	0.825678	15.1388	0.904523	0.714286	8730	48.5	50	37	59
1	9	21	73.2222	375.178	45	1.42858	0.714145	7.5696	0.918367	0.714286	3366	74.8	74	56	94
1	9	22	77.3137	227.052	153	1.46509	0.730839	13.9573	0.921687	0.75	4472	29.2288	30	24	35
1	9	23	76.2075	362.453	53	1.14816	0.492694	8.21472	0.913793	0.736111	3514	64.3019	69	50	80
1	9	24	83.9479	94.3646	392	1.27679	0.621756	15.6353	0.923077	0.761905	8805	45.8596	47	37	54
1	9	25	96.0609	317.026	315	1.25503	0.604248	32.1005	0.912498	0.746753	4048	35.2	36	27	43
1	9	26	98.0964	190.528	397	1.14484	0.486908	15.8376	0.920561	0.724265	9240	46.9036	48	36	59
1	9	27	96.6796	467.951	103	1.32342	0.658926	11.4518	0.895652	0.72028	4108	39.8835	41	31	49
1	9	28	102.493	121.97	203	2.8942	0.338612	16.0769	0.835391	0.461364	8738	43.0443	42	38	53
1	9	29	100.951	38.6639	122	1.72624	0.815118	12.4634	0.917393	0.622449	3920	32.1311	33	26	39
1	9	30	101.027	99.4591	149	1.84047	0.844726	13.7736	0.925466	0.662222	4349	29.1879	30	22	35
1	9	31	99.8	203.069	130	1.27609	0.621208	12.8455	0.902778	0.714288	4411	33.9308	35	27	41
1	9	32	104.422	439.305	109	1.17019	0.519347	11.7406	0.915968	0.758844	4199	38.5229	39	30	46
1	9	33	109.131	54.3347	199	1.51352	0.75064	15.9177	0.929907	0.765385	8907	44.7588	47	38	54
1	9	34	109.456	341.911	90	1.30509	0.642565	10.7047	0.918367	0.692308	3953	43.9222	45	35	53
1	9	35	110.122	343.843	115	1.22263	0.575349	32.1005	0.912698	0.746753	4134	35.9478	37	28	44
1	9	36	116.918	98.9877	81	1.22391	0.58217	10.1354	0.920435	0.838182	4046	49.9506	51	42	59
1	9	37	116.502	401.541	98	1.22722	0.57967	11.1704	0.899083	0.742424	3924	40.0408	41	33	48
1	9	38	123.37	294.65	200	1.59791	0.779969	15.9577	0.917431	0.714286	9236	46.18	47	35	58
1	9	39	128.98	131.02	99	1.28036	0.630508	11.2272	0.9	0.692308	4166	42.0808	42	33	51
1	9	40	127.437	444.757	103	1.71763	0.813046	11.4518	0.895652	0.686667	4065	39.466	41	31	48
1	9	41	133.758	396.913	277	1.47512	0.735146	18.78	0.923333	0.70844	9756	35.2202	37	26	43
1	9	42	136.811	428.693	101	1.15959	0.506271	11.3101	0.90991	0.765152	4129	40.8812	42	33	48
1	9	43	139.48	254.46	387	1.82919	0.837336	15.4304	0.916667	0.467857	9071	48.508	50	37	61
1	9	44	141.077	492	106	1.45378	0.72584	11.6174	0.929825	0.741258	4167	39.3113	40	31	48
1	9	45	146.702	221.56	86	1.39597	0.697744	10.3418	0.903226	0.777778	4112	48.9524	49	36	61
1	9	46	150	476.133	106	1.21719	0.570307	11.6174	0.898305	0.781258	4275	40.3302	42	30	49
1	9	47	161.276	449.352	105	1.38249	0.690631	11.5424	0.913043	0.681818	4157	39.5905	41	32	48
1	9	48	171.034	329.937	284	2.56996	0.94161	19.0158	0.890282	0.473333	13145	46.2852	48	36	56
1	9	49	165.634	211.868	103	1.36598	0.481225	11.3101	0.885965	0.704291	4301	42.5842	44	33	52
1	9	50	165.733	507.368	122	1.21828	0.571176	12.4636	0.910448	0.72619	4098	33.5902	35	27	41
1	9	51	169.352	42.2211	199	1.84139	0.839689	15.9177	0.921294	0.676871	8842	44.4322	46	34	54
1	9	52	182.642	191.49	349	1.96965	0.861532	21.0199	0.894872	0.606951	19671	56.3639	59	44	68
1	9	53	187.426	251.442	329	1.36425	0.680225	12.8159	0.902098	0.671875	4487	34.7829	35	28	42
1	9	54	187.183	418.358	309	1.76008	0.823099	11.7406	0.886179	0.598901	3828	35.1193	36	27	43
1	9	55	191.155	51.9	220	1.46325	0.730036	16.7366	0.932203	0.785714	8915	40.5227	41	31	50
1	9	56	192.106	128.697	142	1.35316	0.673698	13.4462	0.928105	0.728205	5924	41.7183	44	33	51
1	9	57	193.019	443.712	104	1.69436	0.807771	11.5073	0.896552	0.615385	3752	36.0769	36	28	44
1	9	58	194.321	366.037	187	2.07196	0.875821	15.4304	0.912195	0.636054	4596	24.5775	25	18	31
1	9	59	199.016	484.556	126	1.26109	0.603268	12.666	0.947368	0.807692	4126	32.746	33	25	40
1	9	60	207.261	178.63	46	1.54709	0.763022	7.65304	0.836364	0.638889	6268	136.261	142	108	166
1	9	61	209.325	373.431	160	2.41035	0.910505	14.213	0.914286	0.634921	4498	28.1125	28	21	36
1	9	62	211.169	59.5135	148	1.79868	0.831208	13.7273	0.89697	0.685185	4687	31.6689	33	25	38
1	9	63	217.664	231.645	152	1.81213	0.833953	13.9116	0.926829	0.8	4545	29.9013	31	23	36
1	9	64	219.311	73.4662	148	1.62943	0.78953	13.7273	0.942875	0.791444	6274	42.3919	43	33	51
1	9	65	217.944	99.6151	144	1.47822	0.736453	13.5406	0.917197	0.705882	4420	30.6944	32	24	37
1	9	66	222.402	498.069	102	1.44952	0.723919	11.3361	0.894737	0.662338	3850	37.7451	39	30	47
1	9	67	227.369	450.393	84	1.22251	0.57523	10.3418	0.933333	0.763636	3723	44.3214	46	36	57
1	9	68	232.114	280.992	123	1.40951	0.704741	12.5143	0.924812	0.745455	4194	34.0976	35	26	42
1	9	69	235.639	393.325	166	2.34611	0.904612	14.5361	0.907104	0.542484	4447	26.7892	27	20	33
1	9	70	234.904	159.211	209	1.50663	0.747971	16.3128	0.93722	0.765568	9114	43.6077	46	35	54
1	9	71	236.104	297.94	134	1.48761	0.740352	13.0619	0.911565	0.761164	4302	32.1045	33	25	39
1	9	72	237.048	27.3942	104	1.59005	0.777477	11.5073	0.912281	0.683333	3989	38.3558	40	31	47
1	9	73	242.184	51.7053	207	1.81456	0.83444	16.2365	0.907895	0.723776	7036	33.9903	33	25	43
1	9	74	242.764	78.9857	140	1.39685	0.698305	13.3512	0.903226	0.666667	4272	30.5143	31	23	38
1	9	75	245.917	439.287	108	1.58813	0.776882	11.7265	0.931034	0.8	4021	37.2315	38	29	45
1	9	76	252.39	397.256	172	1.71191	0.811652	14.7988	0.900524	0.646617	4653	27.0523	28	21	33
1	9	77	265.139	361.344	294	1.5917	0.778957	19.3477	0.88024	0.665158	5550	32.483	34	24	40
1	9	78	267.579	39.9331	254	1.38602	0.692424	17.9834	0.852349	0.711485	9097	35.815	35	27	43
1	9	79	274.18	118.624	278	1.33653	0.663466	18.8138	0.905537	0.661905	16009	57.5863	60	43	71
1	9	80	277.402	11.082	122	1.34982	0.671749	12.4634	0.924242	0.72619	4155	34.418	36	28	41
1	9	81	285.594	491.97	258	1.62601	0.788526	19.4788	0.814208	0.580897	8476	31.7587	32	24	39
1	9	82	287.241	474.126	278	2.44737	0.912713	18.8138	0.810496	0.620536	9278	33.3741	34	28	40

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1	9	83	283.646	101.811	127	1.6816	0.805787	12.7162	0.913669	0.661458	4625	36.4173	37	29	44
1	9	84	284.665	440.671	173	1.53854	0.759964	14.0415	0.925134	0.750772	7670	44.3351	46	35	54
1	9	85	281.69	289.917	84	1.53456	0.758512	10.3418	0.903226	0.646156	4211	50.131	51	39	62
1	9	86	286.312	80.4481	154	1.13337	0.470446	14.0029	0.916667	0.733333	4526	29.3896	30	23	36
1	9	87	292.989	305.311	90	1.00786	0.393718	10.7047	0.909091	0.743802	4134	45.9333	46	36	56
1	9	88	300.374	47.9346	107	1.37271	0.685058	11.672	0.89916	0.764286	4067	38.0091	39	29	46
1	9	89	302.5	332.755	106	1.53544	0.759839	11.6174	0.938053	0.84327	4700	44.3396	44	36	54
1	9	90	310.561	348.784	148	2.38785	0.908085	13.7273	0.840909	0.528571	8947	60.4527	60	44	74
1	9	91	305.159	364.341	44	1.29753	0.637205	7.48482	0.844154	0.6875	3760	85.4545	88	65	106
1	9	92	313.183	388.246	224	2.05865	0.874094	16.888	0.914286	0.59893	9246	41.2768	43	32	50
1	9	93	312.337	242.253	359	2.13587	0.883626	21.3797	0.810384	0.569841	19817	55.2006	56	42	69
1	9	94	315.082	322.148	182	1.48417	0.738934	15.2227	0.928571	0.738842	9474	52.0549	55	41	64
1	9	95	322.65	51.4724	163	1.82189	0.835902	14.4062	0.910615	0.639216	4364	26.773	28	20	32
1	9	96	327.5	256.405	150	1.28078	0.624816	14.1835	0.923977	0.752381	4988	31.5696	33	25	38
1	9	97	343.743	422.568	241	1.64439	0.753838	17.5172	0.895911	0.634211	9916	41.1152	42	31	51
1	9	98	342.931	46.3444	151	1.33418	0.661977	13.8658	0.920732	0.725962	4249	28.1391	29	22	34
1	9	99	344.143	139.607	28	1.12468	0.460686	5.97082	0.875	0.666667	5659	202.107	215	166	238
1	9	100	352.064	283.674	173	1.60966	0.783613	14.8415	0.935135	0.758772	4659	27.1618	28	21	33
1	9	101	356.062	396.634	145	1.37417	0.685884	13.5875	0.929487	0.74359	8423	58.0897	60	47	69
1	9	102	358.12	38.9816	217	2.20638	0.899318	16.6221	0.90195	0.645833	4595	21.1751	23	17	24
1	9	103	358.408	69.4079	76	1.72377	0.576432	9.83698	0.844118	0.690909	7205	94.8026	99	76	114
1	9	104	358.364	137.455	11	1.13876	0.478395	3.74241	0.785714	0.6875	210	19.0909	19	14	22
1	9	105	370.203	313.176	148	1.83856	0.839143	13.7273	0.89697	0.621849	4306	29.0946	30	22	35
1	9	106	373.012	202.324	166	2.28347	0.89901	14.5381	0.922222	0.65873	4596	27.6867	29	21	34
1	9	107	377.777	170.057	404	1.31694	0.650698	22.6801	0.930874	0.706294	20716	51.2772	52	38	64
1	9	108	384.198	243.899	328	1.6615	0.734714	20.4358	0.931818	0.788462	12022	36.6524	37	27	46
1	9	109	375.638	462.116	69	1.52244	0.75403	9.37302	0.894104	0.427273	3544	51.3623	52	41	62
1	9	110	383.181	491.839	155	1.6691	0.800655	14.0482	0.922619	0.645833	5726	36.9419	38	29	45
1	9	111	392.202	297.197	213	1.27925	0.623644	16.4682	0.926087	0.739583	6337	29.7512	30	22	38
1	9	112	391.713	407.231	195	1.80111	0.831707	15.757	0.924171	0.714286	9007	46.1897	48	35	58
1	9	113	404.037	455.366	82	1.57912	0.773937	10.2179	0.911111	0.759259	3565	48.1098	51	38	59
1	9	114	418.317	476.067	60	1.26455	0.595299	8.74039	0.849565	0.666667	4998	83.3	85	63	101
1	9	115	430.121	389.29	124	1.23756	0.589125	12.5651	0.905109	0.738095	4216	34	35	26	41
1	9	116	434.672	217.156	250	1.45538	0.72655	17.8412	0.93305	0.730994	9416	37.664	39	30	46
1	9	117	430.222	486.351	72	1.21487	0.567797	9.57461	0.878049	0.72	3740	51.9444	52	41	61
1	9	118	438.356	245.69	323	3.93418	0.967156	20.2795	0.913602	0.419481	11119	34.4241	36	27	42
1	9	119	444.212	341.171	316	2.27398	0.898116	20.0585	0.863388	0.548611	17939	56.769	58	43	71
1	9	120	441.239	288.096	230	1.75024	0.870706	17.1127	0.927419	0.684524	9461	41.1348	42	31	51
1	9	121	442.21	57.6643	143	1.34234	0.667091	13.4935	0.922581	0.744792	8260	57.7622	60	45	70
1	9	122	441.885	437.769	52	1.40181	0.700796	8.13686	0.896552	0.742857	3533	67.9423	70	53	81
1	9	123	450.907	139.683	161	2.22932	0.893749	14.3175	0.92	0.731818	4391	27.2733	27	20	35
1	9	124	453.118	426.193	119	1.29823	0.637708	12.3092	0.901515	0.708333	4146	34.8403	36	27	42
1	9	125	452.477	462.156	128	1.1575	0.502605	12.7662	0.920863	0.757396	4293	33.5391	35	26	40
1	9	126	455.773	440.5	44	1.23615	0.58786	7.48482	0.88	0.785714	3328	75.6364	77	62	90
1	9	127	476.277	407.954	130	1.41595	0.709952	12.8655	0.915493	0.714286	7021	54.0077	56	44	66
1	9	128	479.27	387.828	152	1.48858	0.740748	13.9116	0.853933	0.633333	4951	32.5728	33	24	40
1	9	129	479.157	215.248	121	1.27349	0.61919	12.4122	0.902985	0.735976	4343	35.8926	37	28	43
1	9	130	480.898	10.1837	49	1.4708	0.733302	7.89865	0.907407	0.777778	5891	120.224	122	92	147
1	9	131	485.018	132.565	169	1.6403	0.792675	14.6255	0.933333	0.777778	4290	25.5357	26	20	32
1	9	132	488.025	230.544	160	1.36575	0.68103	14.273	0.946746	0.764314	8081	50.5063	53	41	62
1	9	133	489.527	41.2565	165	1.47325	0.73435	14.4943	0.911602	0.705128	4206	25.4909	27	20	31
1	9	134	488.927	311.65	191	1.39613	0.697829	15.5945	0.927186	0.702206	9350	48.9529	50	38	60
1	9	135	493.91	94.3349	212	2.14811	0.885034	16.4294	0.921739	0.658385	7500	35.3774	38	29	41
1	9	136	495.135	339.708	192	1.32749	0.657674	15.6353	0.932039	0.761905	8831	45.9948	48	36	55.
1	9	137	497.039	369.388	178	2.12112	0.881893	15.0345	0.92228	0.706349	6413	36.0281	38	27	44

Page 16 of 16

EV Table 2.doc

Example of the summary output of AnalyseDNA.m program
(summary for 10 3 by 3 montage images)

1	1187	163.812	79.3918	1.59849	0.398735	0.726461	0.137996	14.0612	3.315	0.905327	0.0350365	0.701248	0.075176	6649.26	3496.95	41.539	18.352	42.9444	18.9393
	32.4103	14.334	50.5906	22.4564															
2	1305	169.034	86.8722	1.60511	0.399942	0.727835	0.138462	14.2634	3.43288	0.906571	0.0344453	0.70177	0.0730289	6786.37	3895.1	42.2416	17.0965	43.8245	17.383
	33.0245	13.3362	51.3608	21.2507															
3	1399	164.57	80.0679	1.38728	0.397933	.0.72552	0.13363	14.0853	3.33904	0.905267	0.0361822	0.702891	0.0720005	6881.08	3525.81	44.3167	20.5918	46.3838	21.2287
	34.8313	16.2062	54.4417	25.1765															
4	1388	172.075	89.7145	1.60189	0.389682	0.727142	0.142518	14.3531	3.61812	0.902766	0.0374254	0.695945	0.0753889	6997.68	4212.87	43.1798	19.8582	44.9561	20.5112
	33.7349	15.9902	52.505	24.193															
5	1448	171.321	90.64	1.58887	0.400493	0.721084	0.141204	14.338	3.65065	0.904152	0.0379804	0.70023	0.0756884	7050.22	4163.04	44.0559	21.4761	45.8522	22.1282
	34.3315	14.6358	53.6367	26.3338															
6	1418	165.142	84.6806	1.60542	0.425512	0.728414	0.133721	14.0974	3.39686	0.904414	0.0362731	0.696204	0.0782855	6843.2	3924.32	44.266	19.3396	45.9485	19.8324
	34.5444	15.2864	53.9055	23.8472															
7	1756	129.864	99.2039	1.53806	0.451022	0.694813	0.185667	11.728	5.27441	0.893311	0.0481729	0.704526	0.0892393	5162.51	4393.11	34.9743	21.1969	36.16	22.0024
	26.8901	17.0423	43.0689	25.6082															
8	1280	173.387	84.1254	1.53381	0.405467	0.723421	0.137599	14.3833	3.40593	0.906384	0.0357179	0.702574	0.0751602	6865.87	3767.48	42.2752	17.2223	43.9781	17.7421
	33.0602	13.7394	51.3961	20.956															
9	1270	166.53	84.5094	1.60361	0.404986	0.72668	0.138548	14.1402	3.47794	0.905208	0.0356298	0.700331	0.0766773	6576.54	4022.38	41.5064	17.8994	43.163	18.2499
	32.3669	13.8149	50.6976	22.2407															
10	1425	159.606	83.618	1.57286	0.400372	0.717147	0.139085	13.8205	3.49536	0.904275	0.0379311	0.702759	0.07572	6587.18	3788.39	44.2141	20.7694	45.8765	21.136
	34.4582	16.3199	53.7958	25.4094															

Page 1 of 1

CLAIMS

What is claimed is:

1. A method of predicting a property of a manipulation of cells based upon a descriptor, said method comprising:

providing a plurality of cells;

manipulating said plurality of cells;

capturing a morphological value from said plurality of cells;

assigning a degree of presence of said morphological value; and

storing said morphological value and said degree of presence;

wherein said descriptor is derived from a first component of a cell and a second component of said cell, said capturing said morphometric value from said plurality of cells comprises determining a relationship between said first component and said second component.

2. The method of claim 1 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a subcelluar structure and an organelle.

3. The method of 1 wherein said step of manipulation occurs in a manner selected from a electrical source, a chemical source, a thermal source, a gravitational source, a nuclear source, a temporal source, and a biological source

4. The method of claim 3 wherein said chemical source is selected from a pharmacological candidate and a drug screening library.

5. The method of claim 1 wherein said morphological value is selected from a count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average gray value, a total gray value, and an optical density.

6. The method of claim 1 wherein said degree of presence is multiple of a quantized value.

7. A computer program product for populating a database with manipulated biological information, said computer program product comprising:

code for providing a plurality of cells in various stages of the cell cycle, said stages of the cell cycle including at least one selected from interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase;

code for manipulating said cells in said various stages of cell cycle development to form a plurality of manipulated cells;

code for capturing an image of said plurality of manipulated cells;

code for determining a descriptor from said image for said manipulated cells;

code for populating a database with said descriptor;

wherein said image includes a first component of a cell and a second component of said cell; and

a computer readable storage medium for holding the codes.

8. The computer program product of claim 7 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a sub-cellular structure and an organelle.

9. The computer program product of claim 7 wherein said image is a digitized representation of said plurality of manipulated cells.

11. The computer program product of claim 9 wherein said digitized representation provides a density value of said plurality of manipulated cells.

11. The computer program product of claim 7 wherein said descriptors comprise numeric or logical values.

12. The computer program product of claim 11 wherein said values further comprises a nucleotide.

13. The computer program product of claim 11 wherein said values further comprises an amino acid letter.

14. A system for capturing images of cells or cell structures, the system comprising:

a cell holder comprising a plurality of sites in a spatial orientation, each of the sites being capable of holding a plurality of cells to be imaged;

an image capturing device coupled to the cell holder, the image capture device being adapted to capture at least one image in at least one of the plurality of sites;

an illumination apparatus comprising a liquid light guide coupled to the plate for highlighting the plurality of cells in a relatively even spatial manner for image capturing purposes;

an image processing device coupled to the image capturing device, the image capturing device being adapted to convert the image into a digital representation; and

a database storage device comprising a database management element coupled to the image capturing device, the database storage device being adapted to retrieve the digital representation of the image from the image processing device and storing the digital representation.

15. The system of claim 14 further comprising a stage comprising a device for moving the cell holder in a spatial direction to traverse across the cell holder in the spatial orientation.

16. The system of claim 14 wherein the illumination apparatus comprises sub-elements, at least one of the sub-elements being positioned away from the image capturing device to prevent a possibility of vibration from the one sub-elements to be transmitted to the image capturing device.

17. The system of claim 14 wherein the digital representation comprises a plurality of regions and objects.

18. The system of claim 14 further comprising a computing device connected between the database storage device and the image processing device.

19. The system of claim 14 wherein the image capturing device comprises a magnification of at least 1X and greater to capture the image of the site.

20. The system of claim 14 wherein the plurality of sites comprises at least 96 sites.

21. The system of claim 14 wherein the liquid light guide characterized as a flexible member that substantially prevents vibration from the an element of the illumination apparatus to be transferred to the image capturing device.

22. The system of claim 14 wherein the spatial direction can be selected from an x-direction, a y-direction, or a z-direction in a Cartesian coordinate system.

23. The system of claim 14 wherein the each of the sites comprises a volume that is sufficient to prevent a solution therein from evaporating in a substantial manner that may influence the image capturing.

24. A method for identifying a mechanism of action for a first compound, the method comprising the steps of:

receiving the first compound;

measuring at least one feature of a cellular phenotype to define a target phenotype;

identifying additional compounds providing a feature similar to the feature identified in the measuring step; and

characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

25. The method of claim 24 comprising the further step of storing the additional compounds and their associated phenotypes in a database.

26. A method for identifying a mechanism of action for a cellular stimulus, the method comprising the steps of:

receiving cells of interest;

measuring at least one feature of the cells to define and quantify a target phenotype;

identifying additional compounds providing a feature similar to the feature identified in the measuring step; and

characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

27. A method for identifying information relevant to at least one of a mechanism of action and cellular activity by utilizing assay data to elucidate a phenotype, the method comprising the steps of:

identifying a target protein;

identifying positive and negative biochemical hits related to the target protein;

defining the target phenotype utilizing the positive and negative hits;

identifying other compounds providing similar features; and

characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

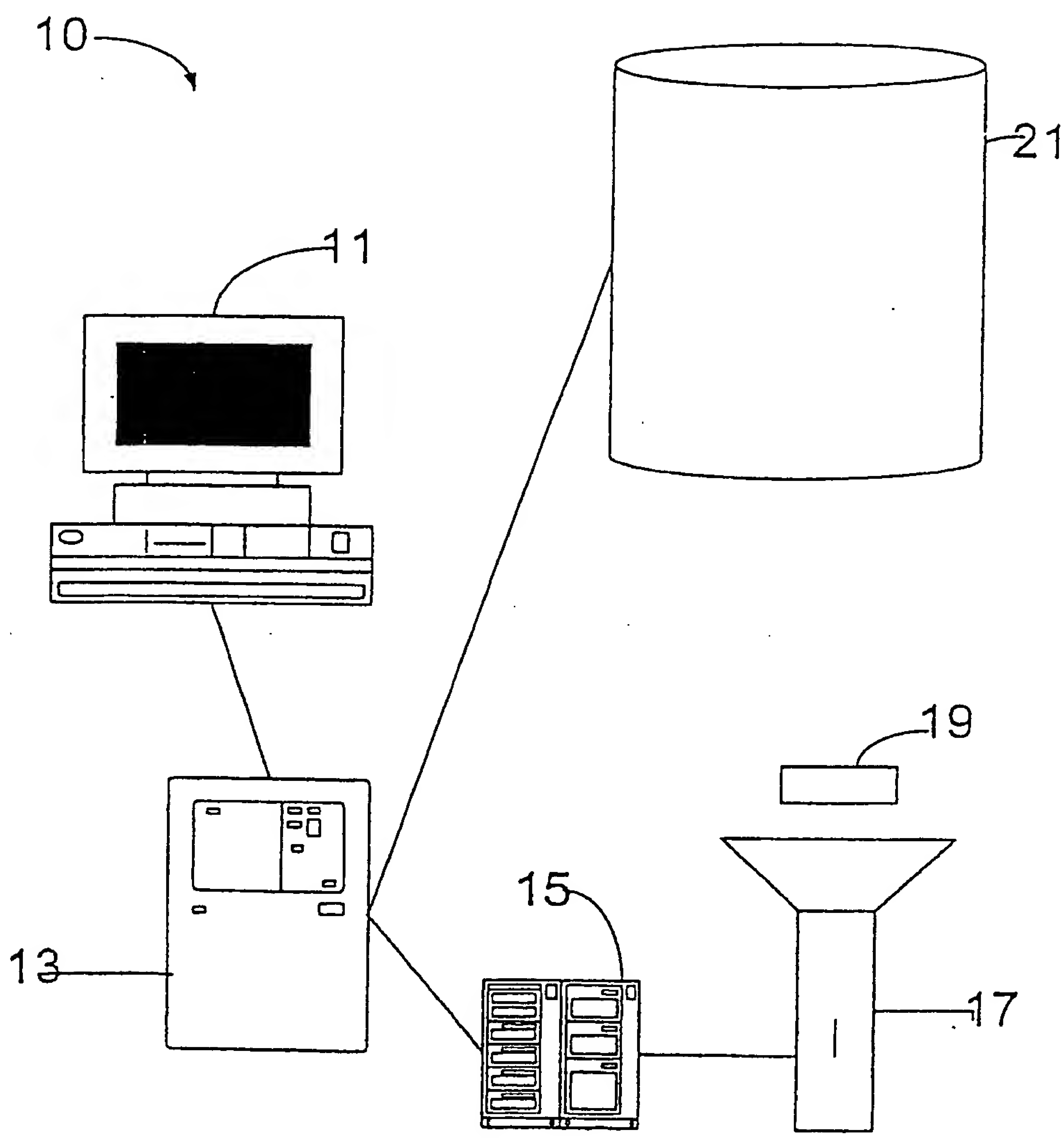


FIG. 1

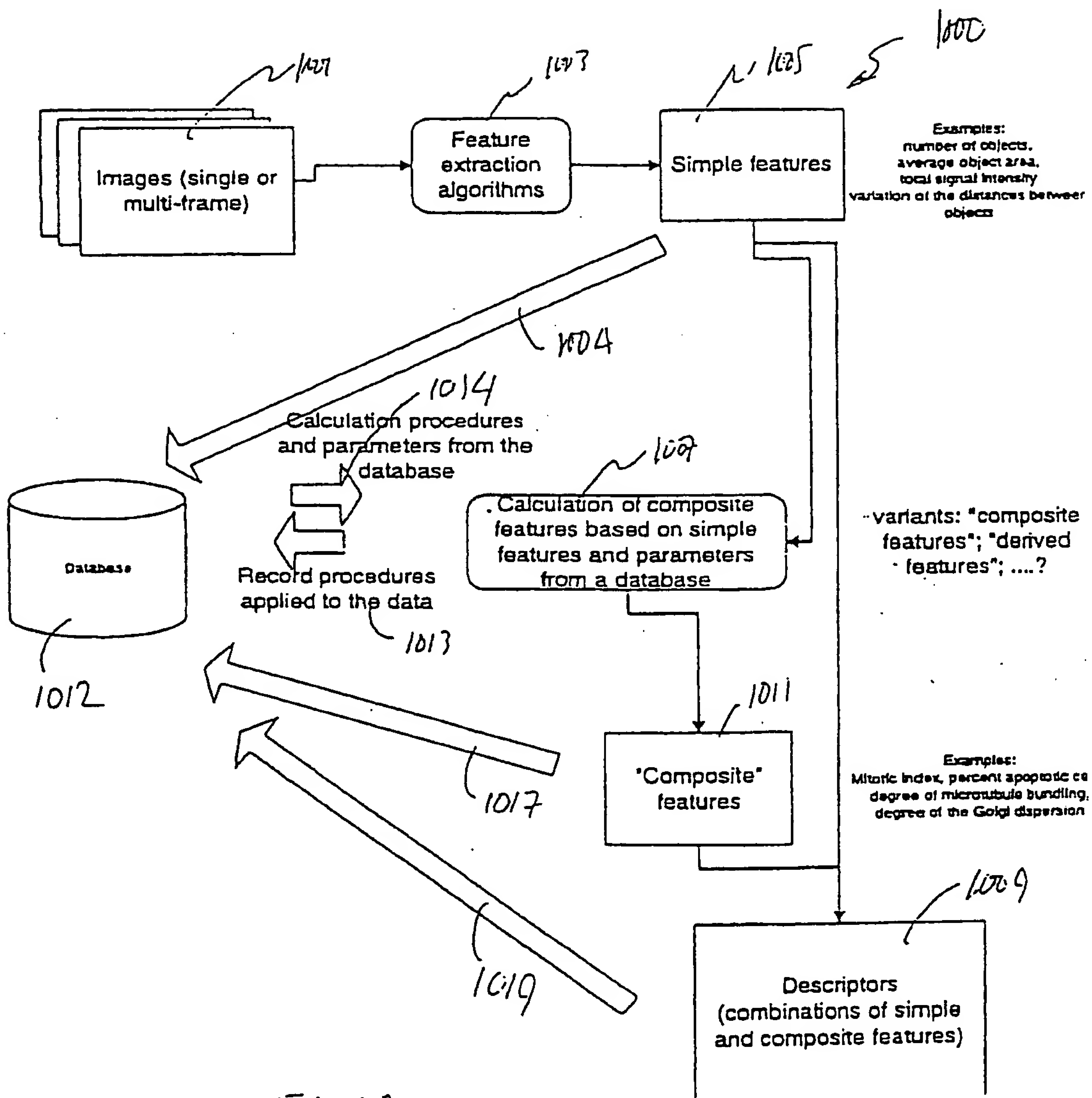

1000
1001
1003
1005
Images (single or multi-frame)
Feature extraction algorithms
Simple features
Examples:
number of objects.
average object area.
total signal intensity
variation of the distances betweer objects
1004
1014
Calculation procedures and parameters from the database
1007
Calculation of composite features based on simple features and parameters from a database
variants: "composite features"; "derived features"; ....?
Database
Record procedures applied to the data
1013
1012
1011
"Composite" features
Examples:
Mitotic index, percent apoptotic ce
degree of microtubule bundling,
degree of the Golgi dispersion
1017
1009
1019
Descriptors (combinations of simple and composite features)

FIG. 1A

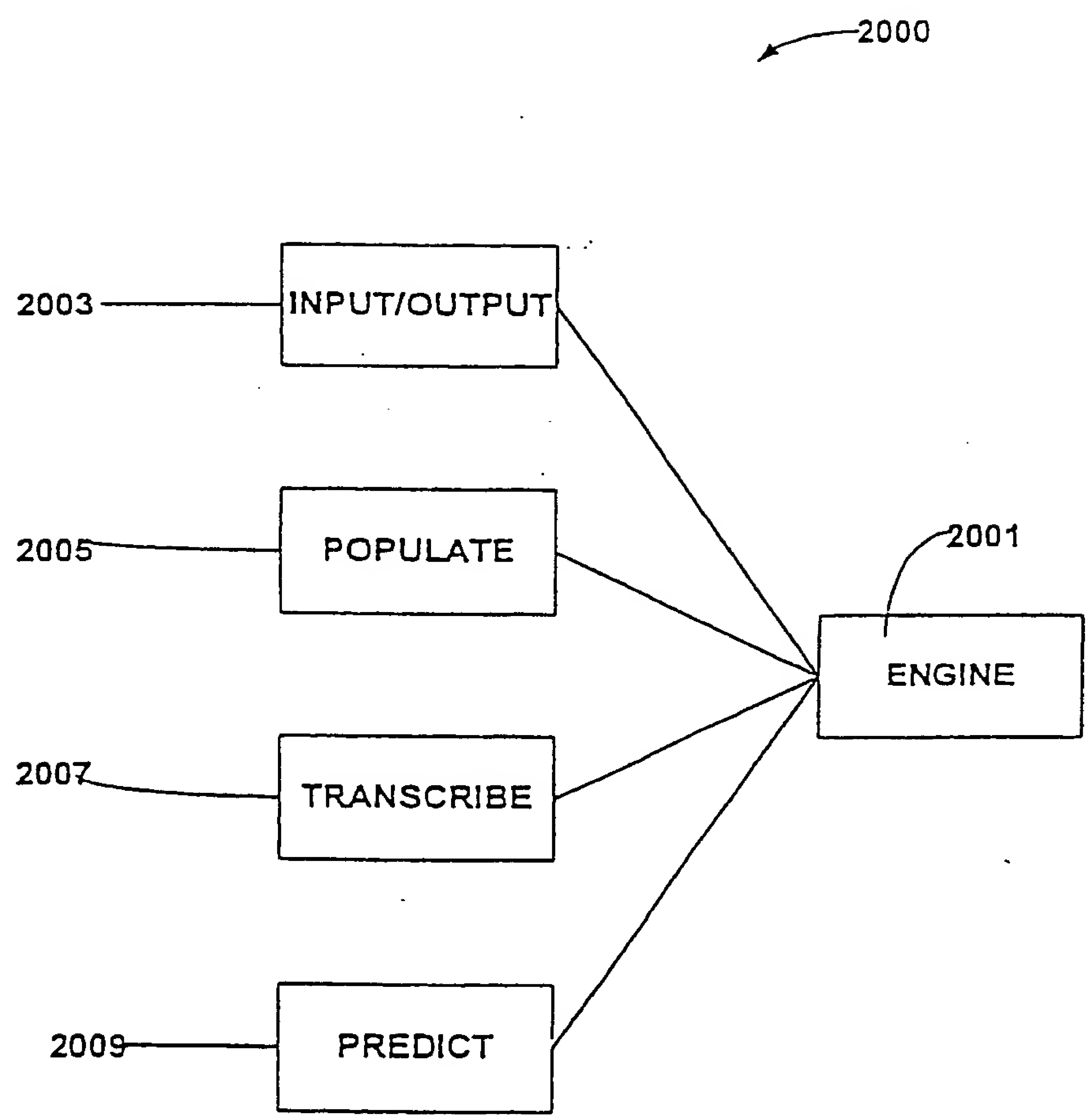

2000
2003
INPUT/OUTPUT
2005
POPULATE
2007
TRANSCRIBE
2009
PREDICT
2001
ENGINE

FIG. 1B

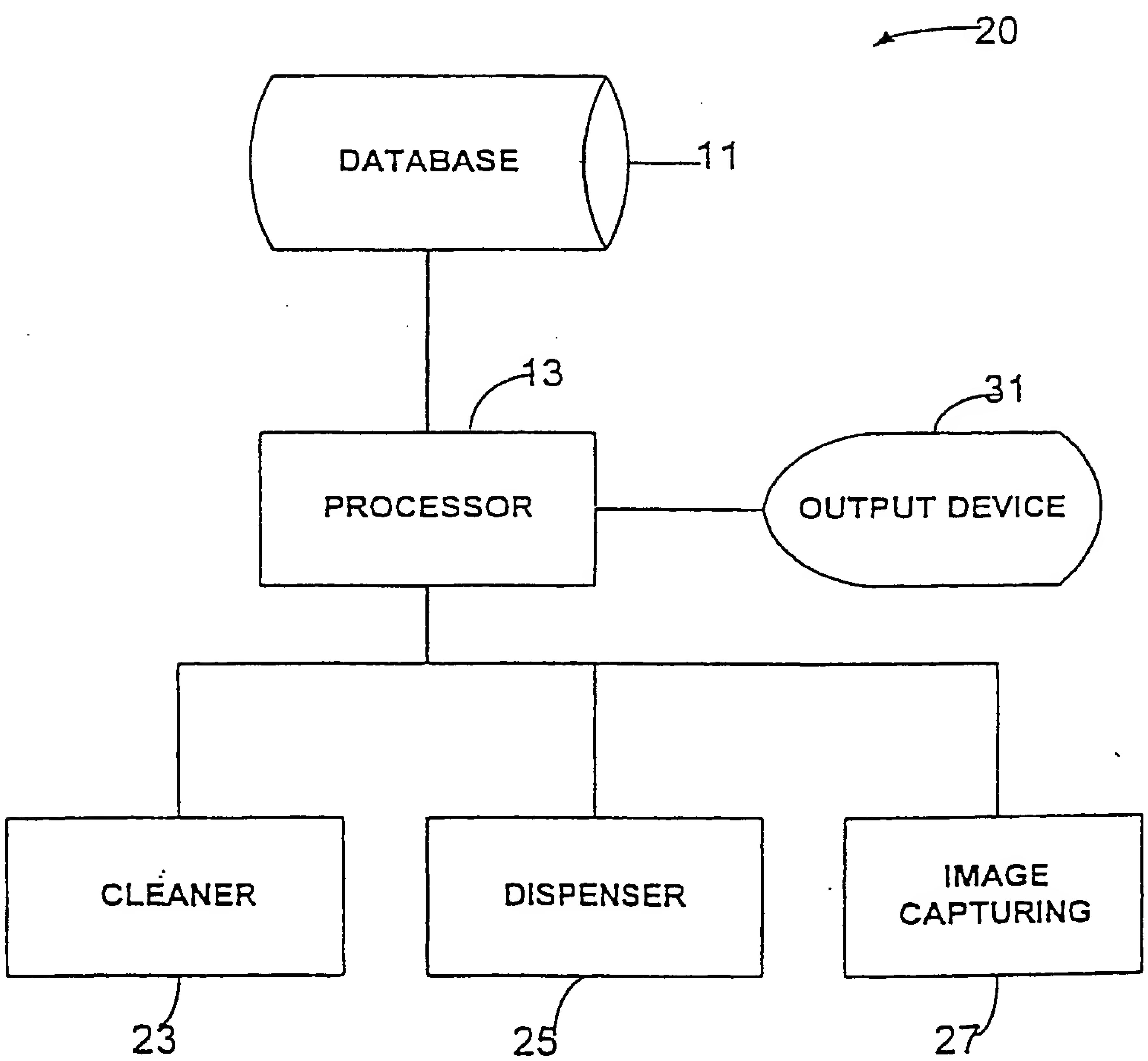

20
DATABASE
11
13
PROCESSOR
31
OUTPUT DEVICE
CLEANER
DISPENSER
IMAGE CAPTURING
23
25
27

FIG. 2

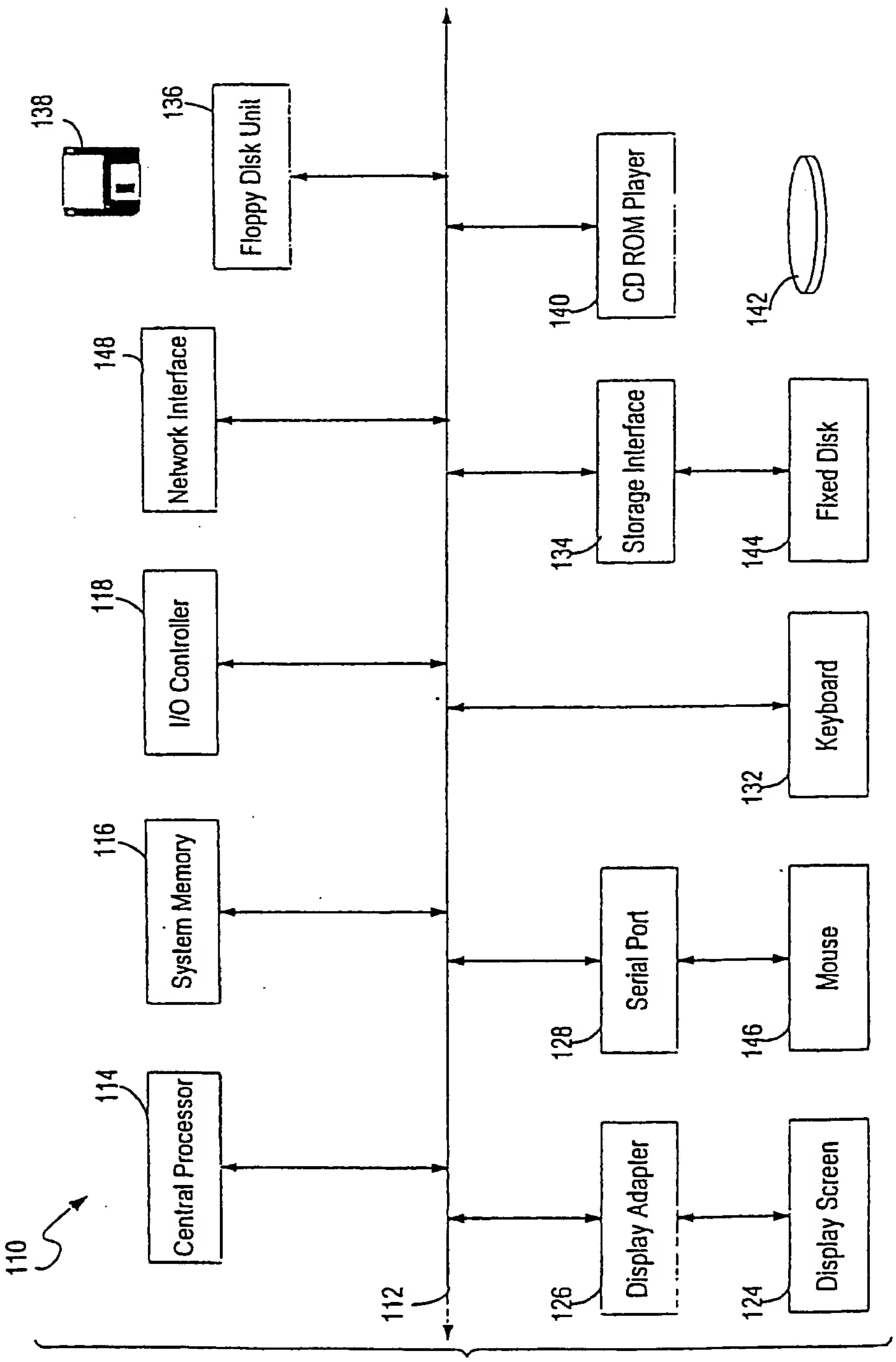

110
Central Processor
114
System Memory
116
I/O Controller
118
Network Interface
148
Floppy Disk Unit
136
138
112
Display Adapter
126
Serial Port
128
Storage Interface
134
CD ROM Player
140
Display Screen
124
Mouse
146
Keyboard
132
Fixed Disk
144
142

Fig 3

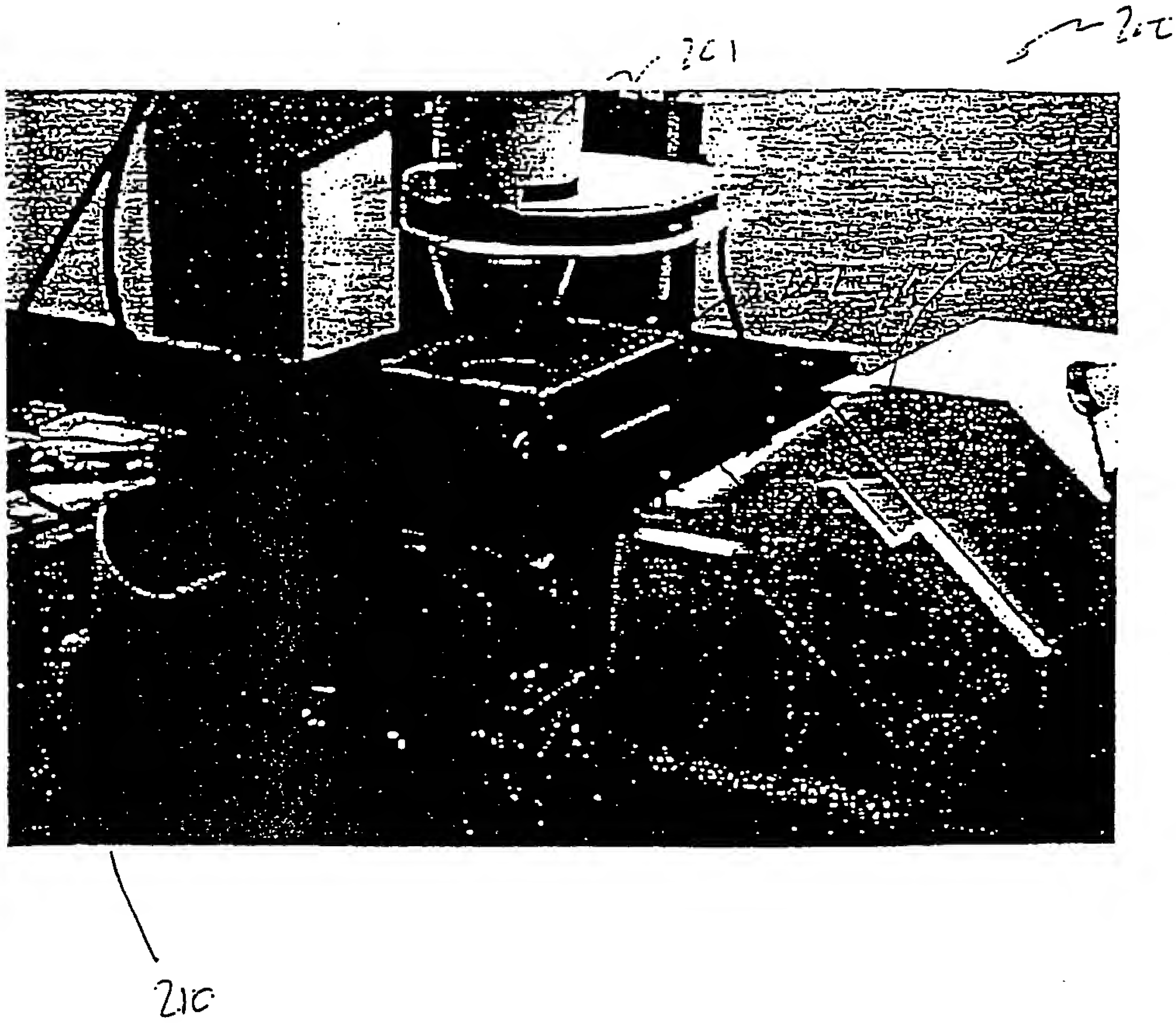


FIG. 4

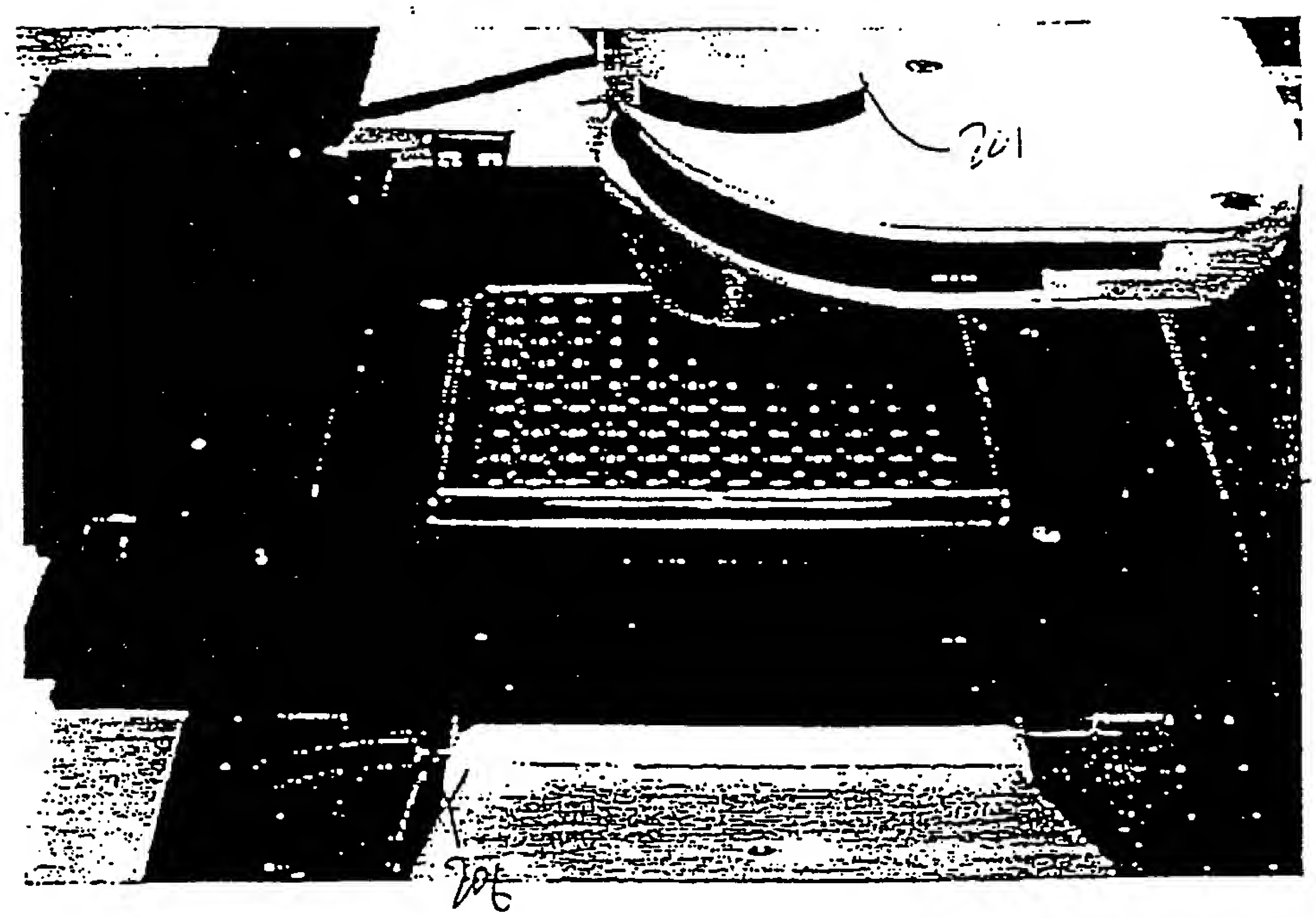


FIG 5

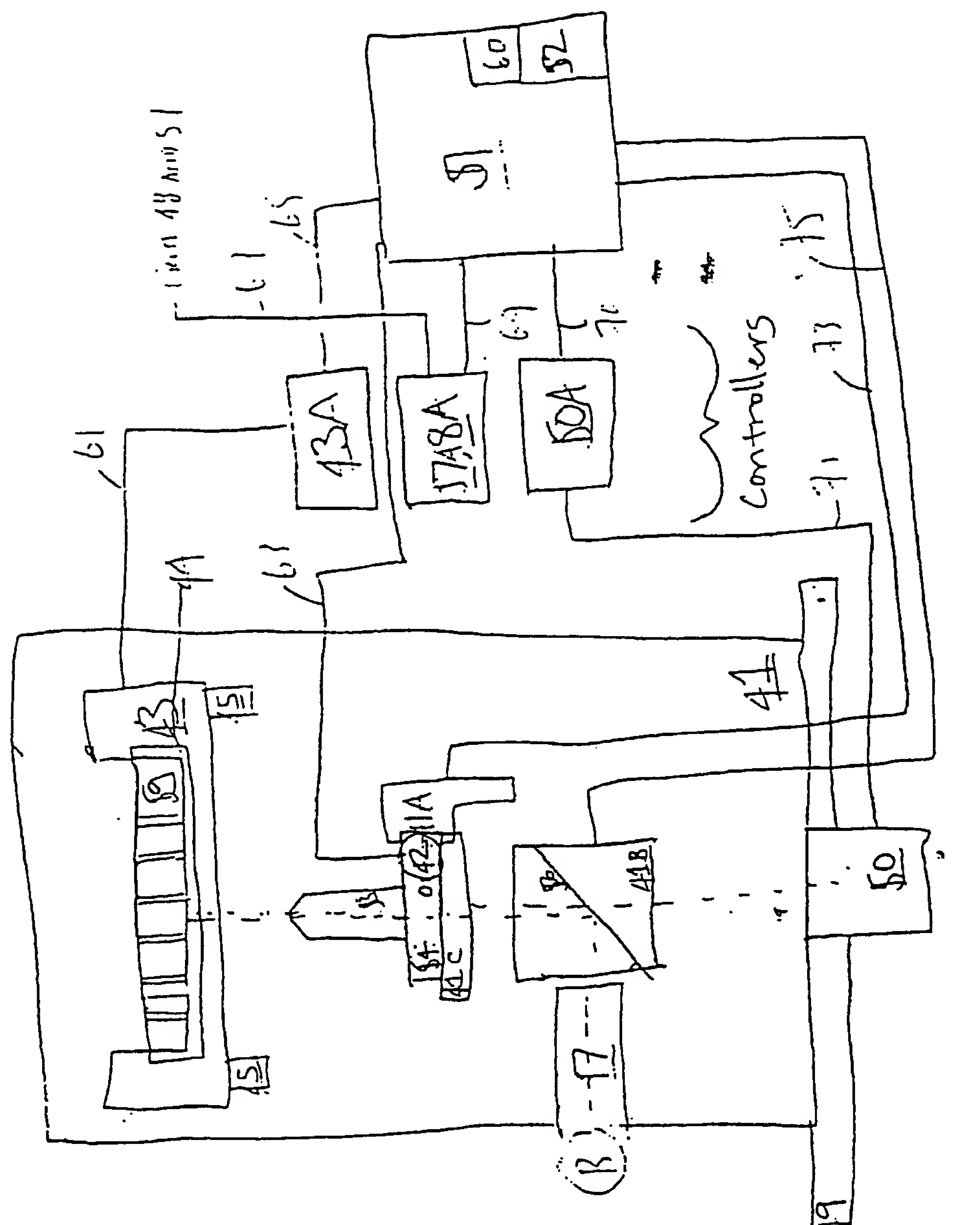


FIG. 5A

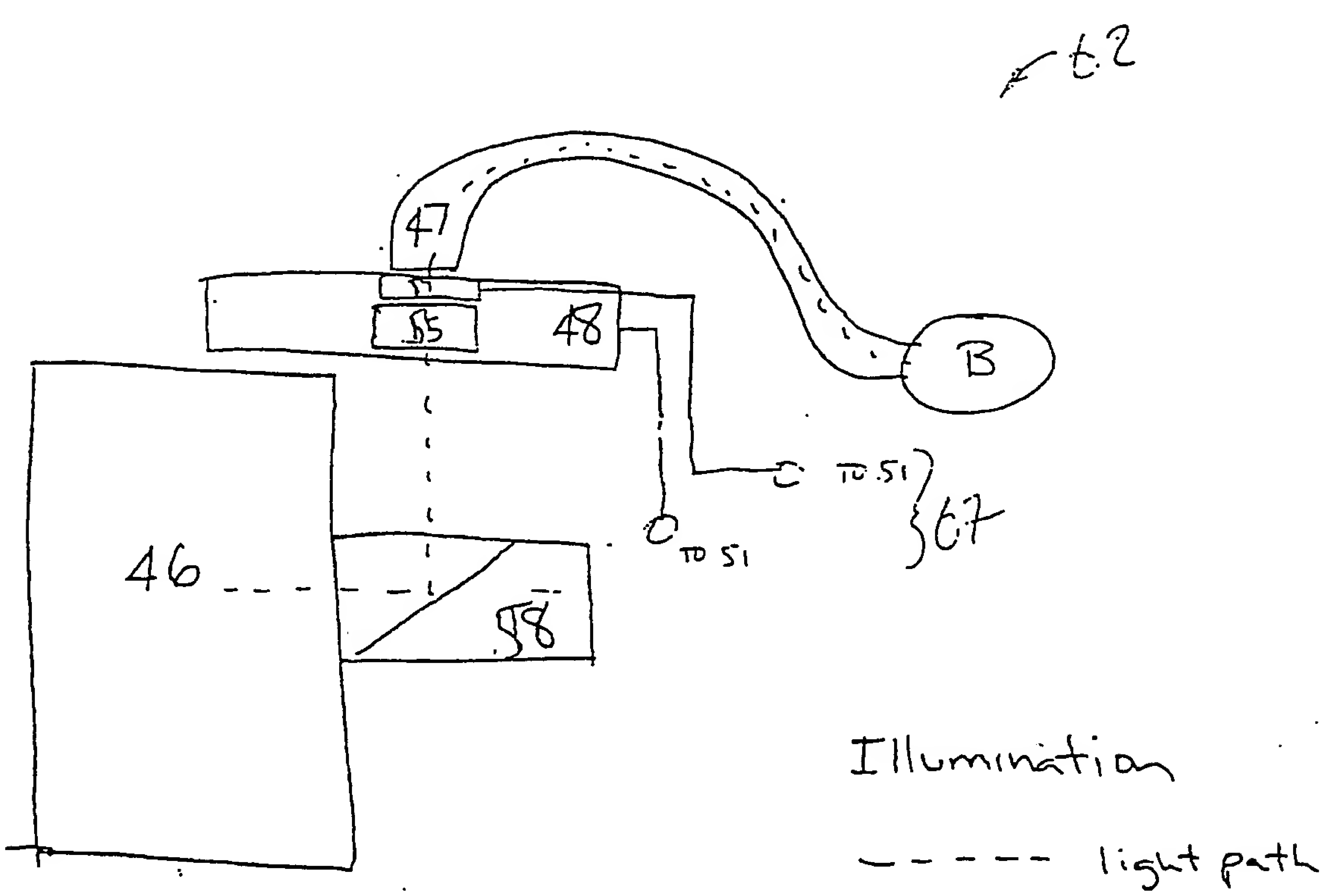


FIG. 5B

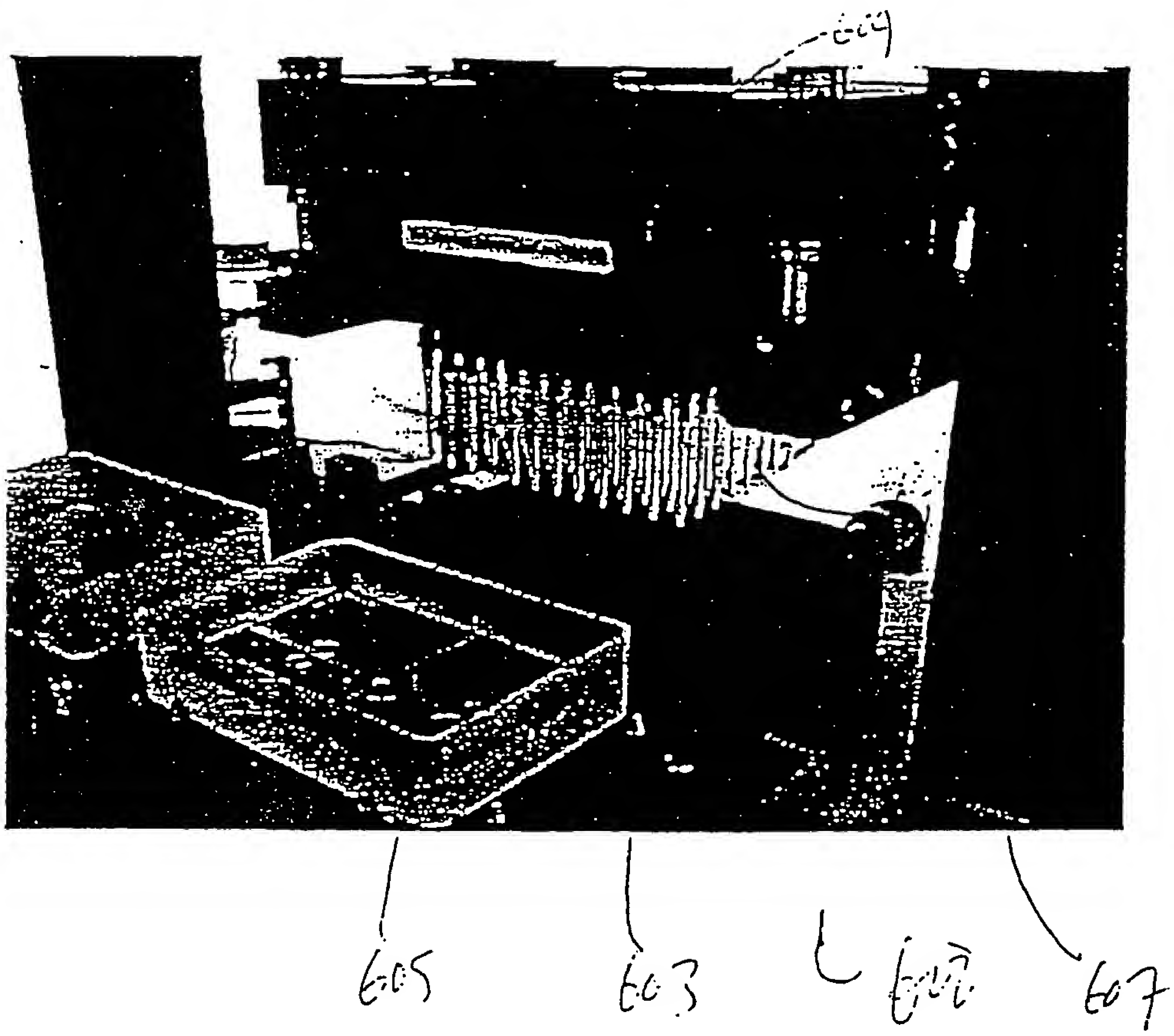


FIG. 6

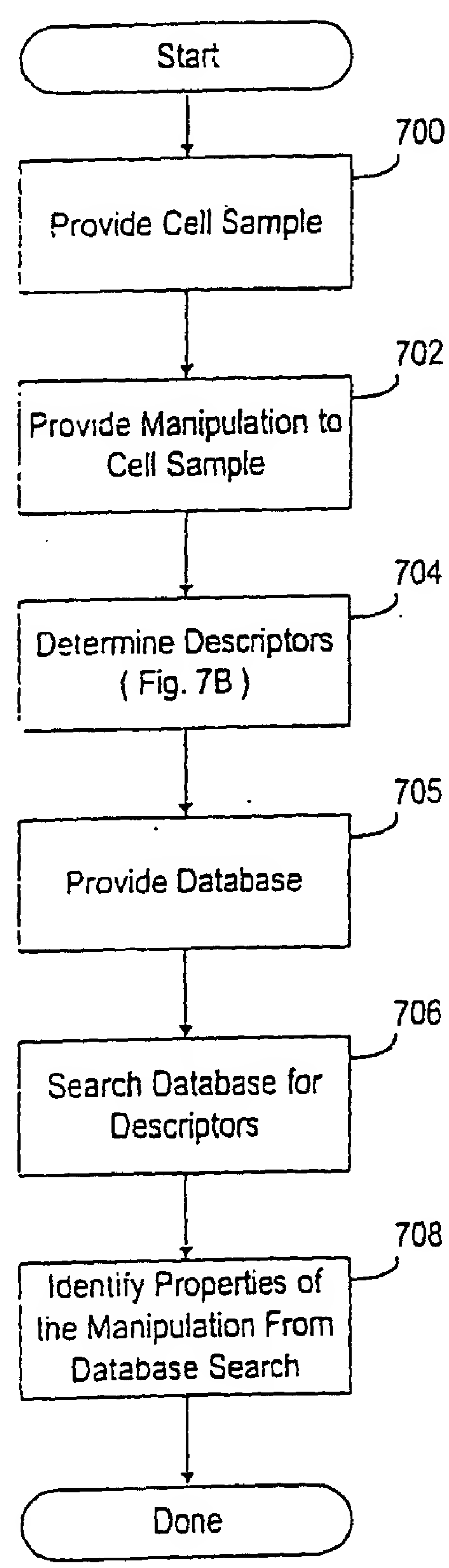

Start
700
Provide Cell Sample
702
Provide Manipulation to Cell Sample
704
Determine Descriptors (Fig. 7B)
705
Provide Database
706
Search Database for Descriptors
708
Identify Properties of the Manipulation From Database Search
Done

Fig. 7A

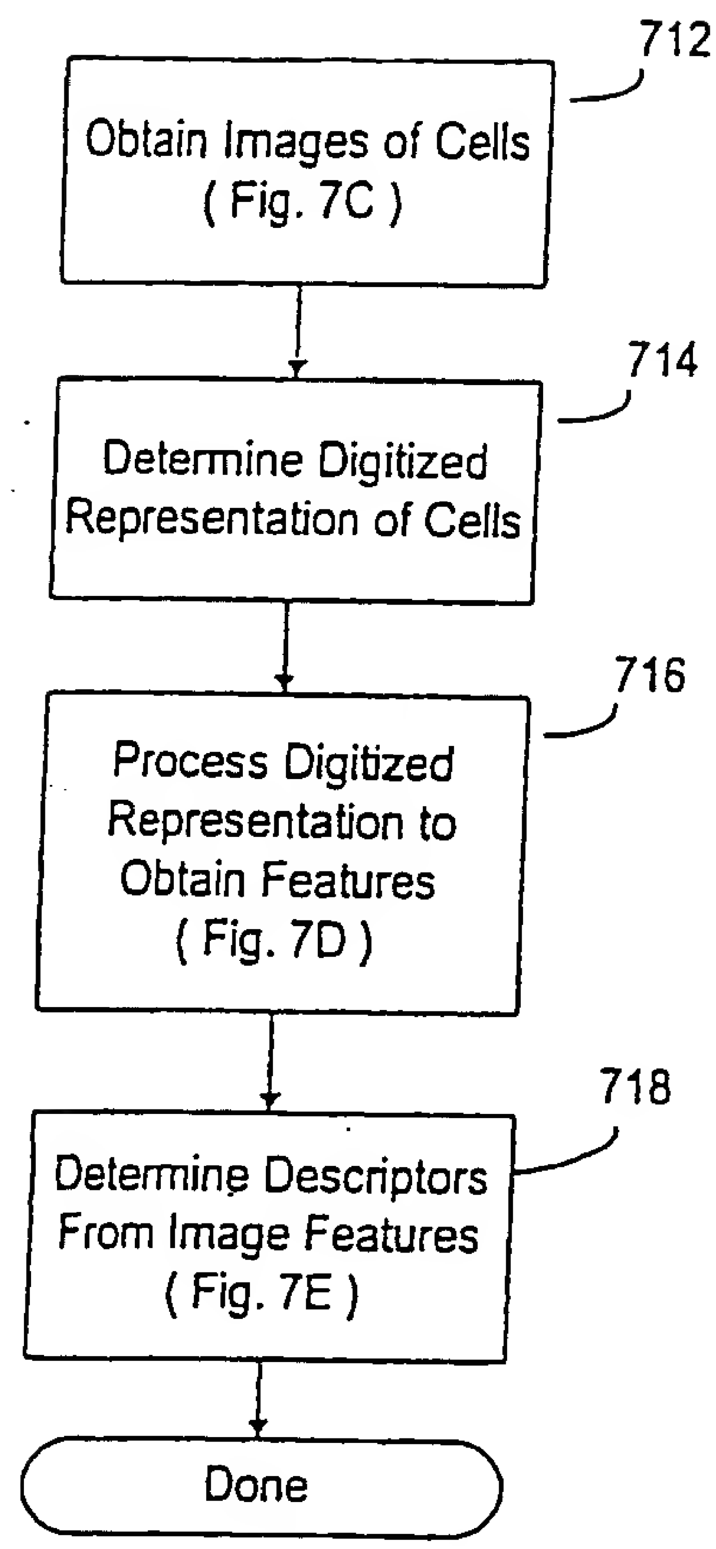


Fig. 7B
Step 704 of Fig. 7A

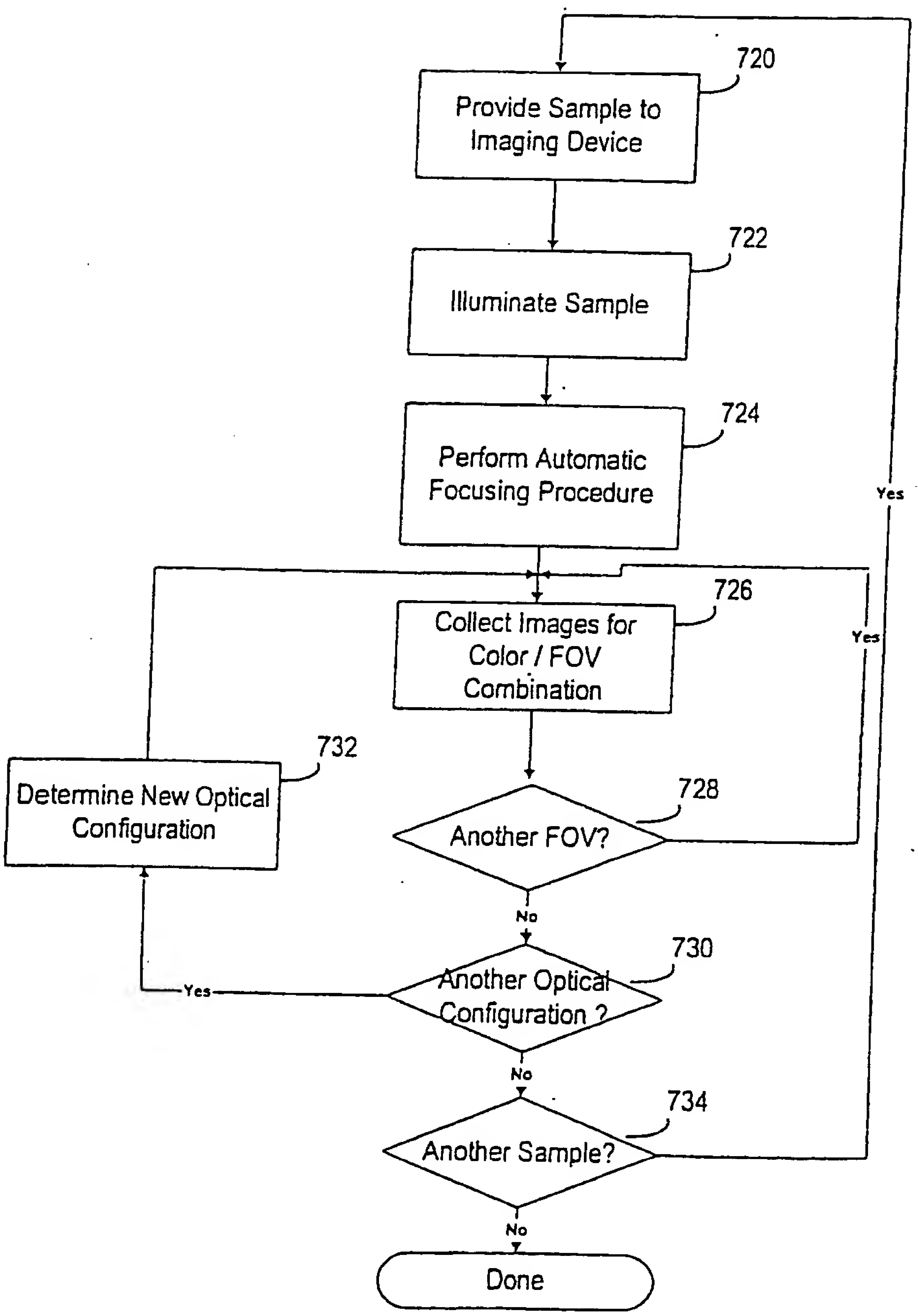


Fig. 7C
Step 714 of Fig. 7B

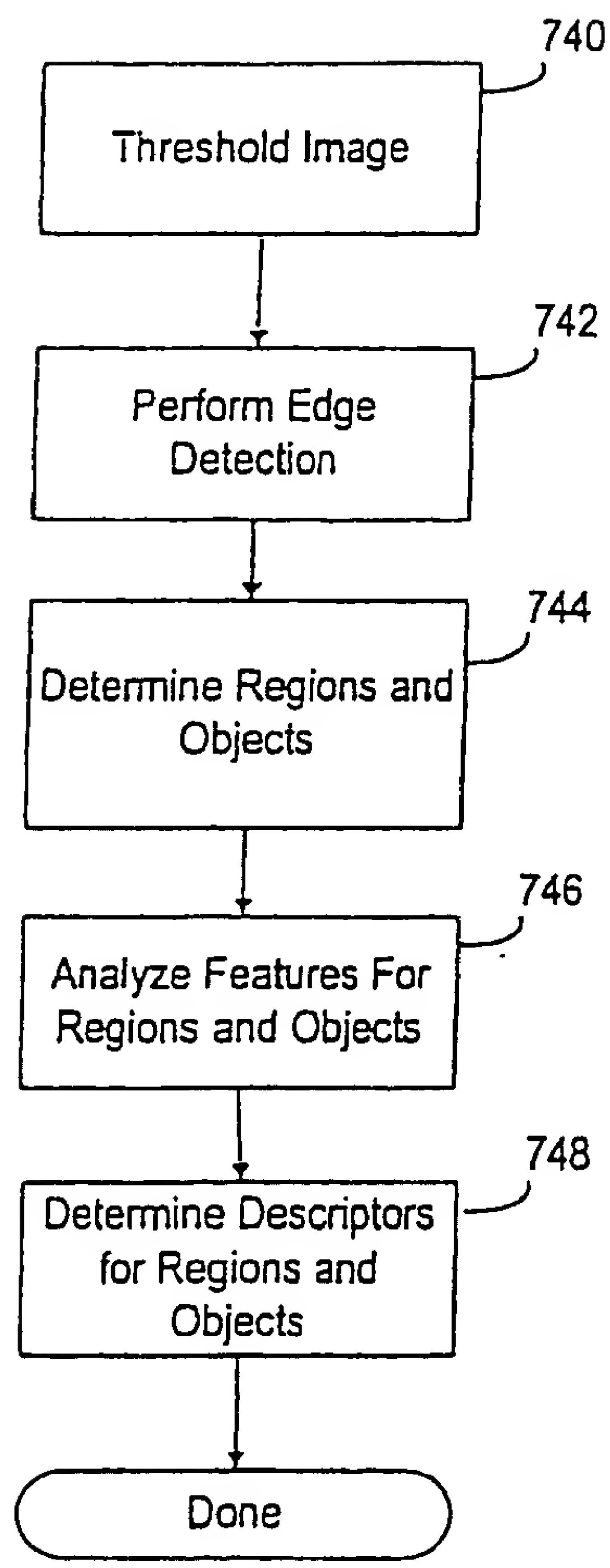


Fig. 7D
Step 716 of Fig. 7B

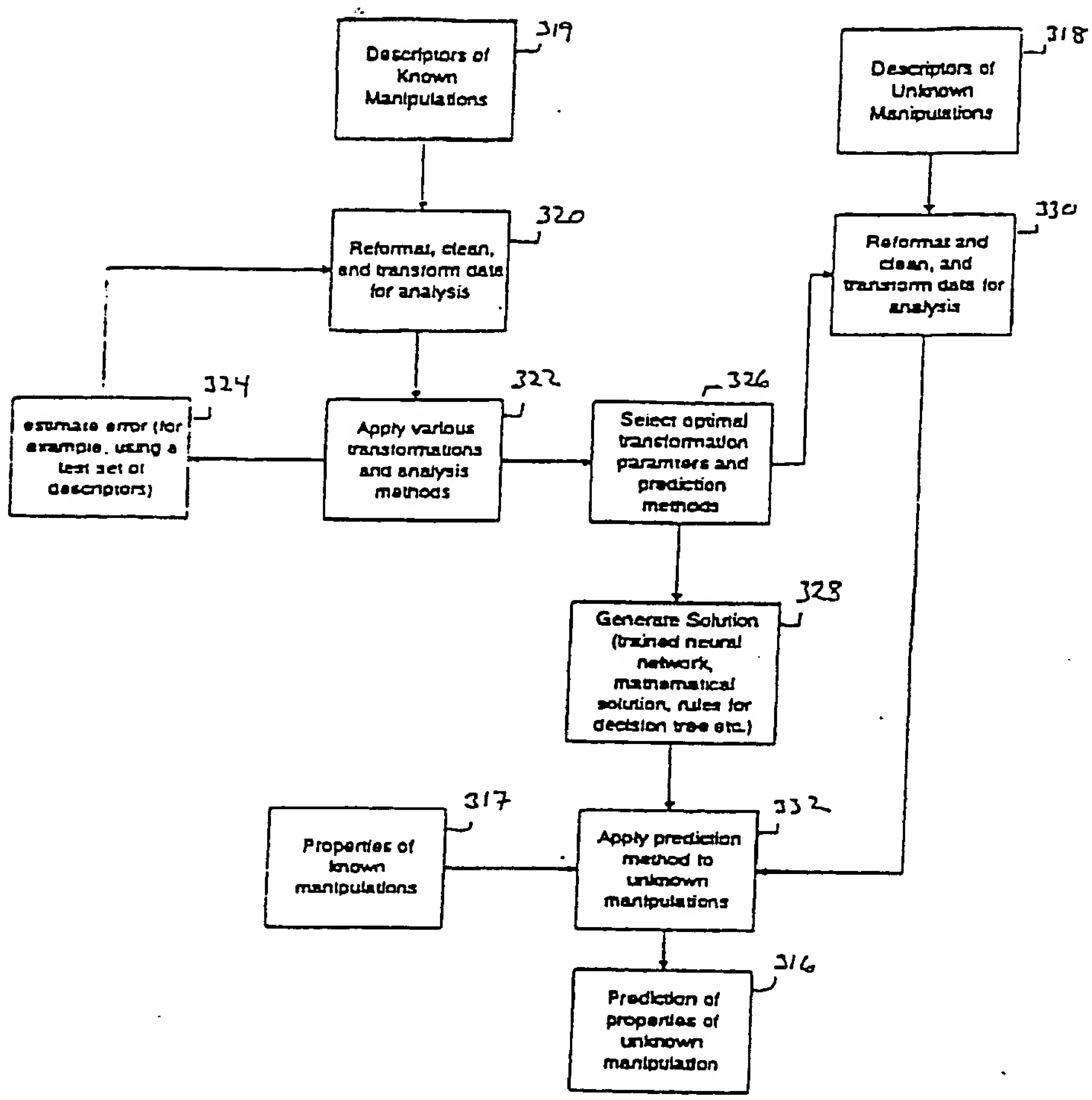

Descriptors of Known Manipulations
319
Reformat, clean, and transform data for analysis
320
estimate error (for example, using a test set of descriptors)
324
Apply various transformations and analysis methods
322
Select optimal transformation parameters and prediction methods
326
Descriptors of Unknown Manipulations
318
Reformat and clean, and transform data for analysis
330
Generate Solution (trained neural network, mathematical solution, rules for decision tree etc.)
328
Properties of known manipulations
317
Apply prediction method to unknown manipulations
332
Prediction of properties of unknown manipulation
316

FIG. 7E

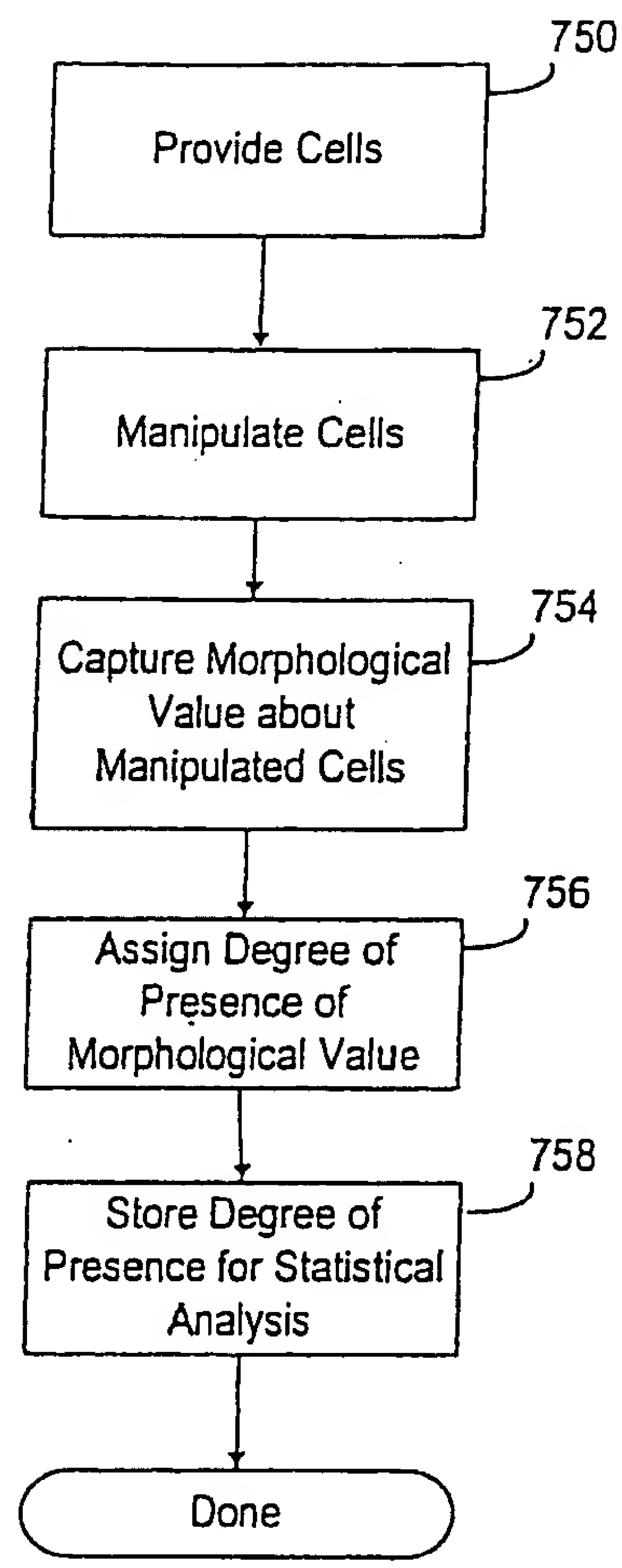

750
Provide Cells
752
Manipulate Cells
754
Capture Morphological Value about Manipulated Cells
756
Assign Degree of Presence of Morphological Value
758
Store Degree of Presence for Statistical Analysis
Done

Fig. 7F

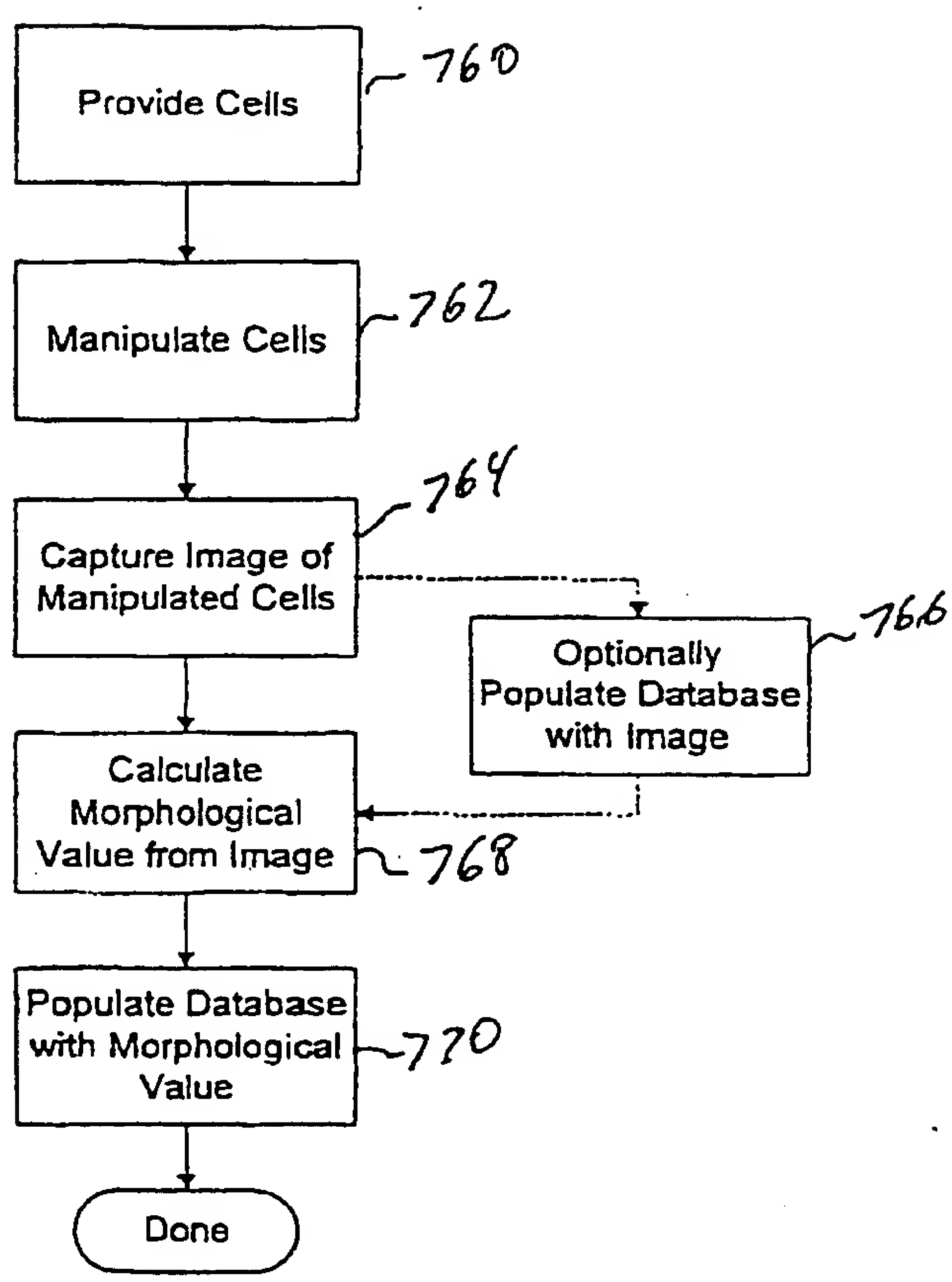

Provide Cells
760
Manipulate Cells
762
Capture Image of Manipulated Cells
764
Optionally Populate Database with Image
766
Calculate Morphological Value from Image
768
Populate Database with Morphological Value
770
Done

Fig 7G

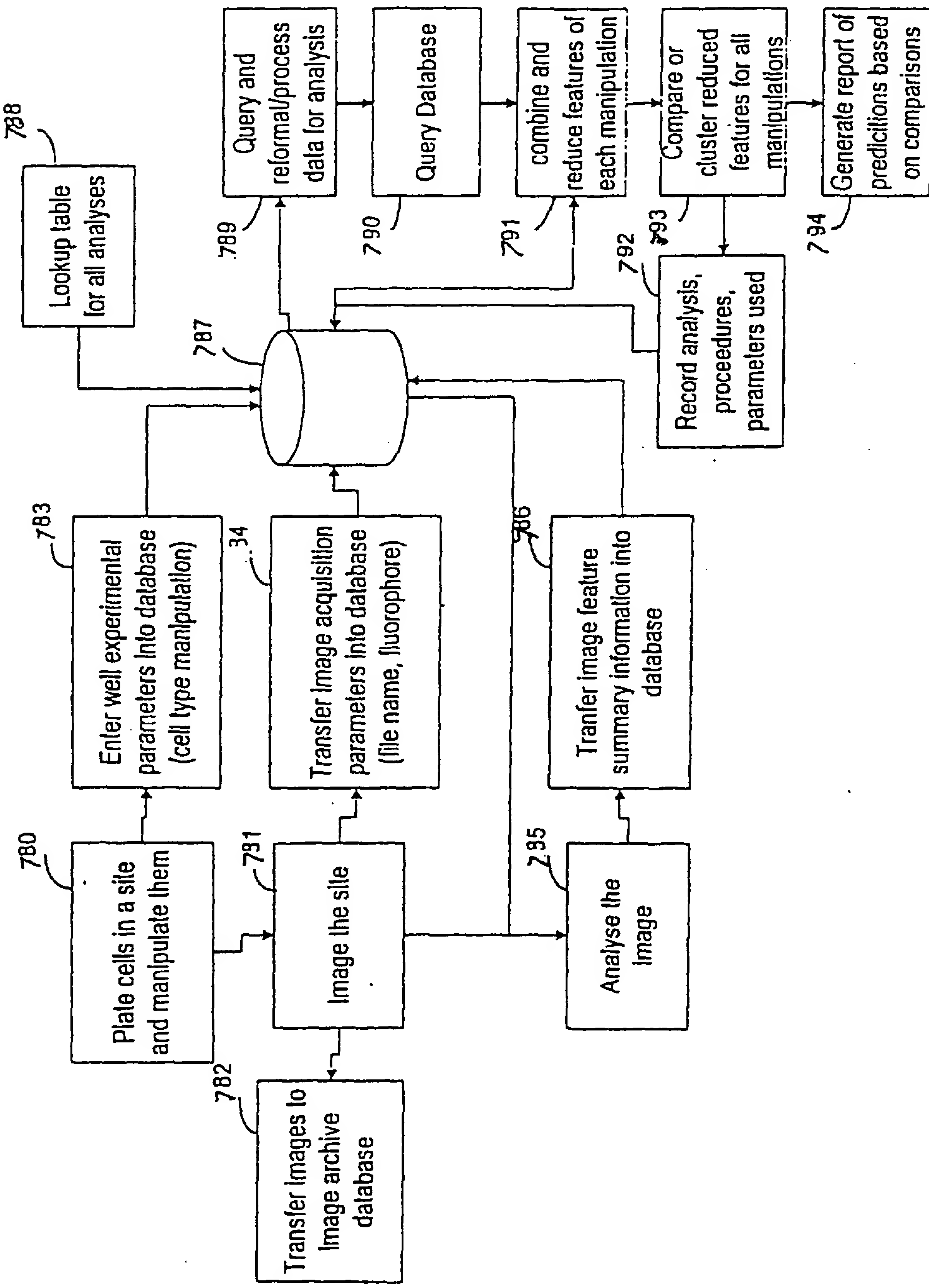

780
Plate cells in a site and manipulate them
781
Image the site
782
Transfer images to image archive database
783
Enter well experimental parameters into database (cell type manipulation)
784
Transfer image acquisition parameters into database (file name, fluorophore)
785
Analyse the image
786
Tranfer image feature summary information into database
787
788
Lookup table for all analyses
789
Query and reformat/process data for analysis
790
Query Database
791
combine and reduce features of each manipulation
792
Record analysis, proceedures, parameters used
793
Compare or cluster reduced features for all manipulations
794
Generate report of predictions based on comparisons

Fig.7H

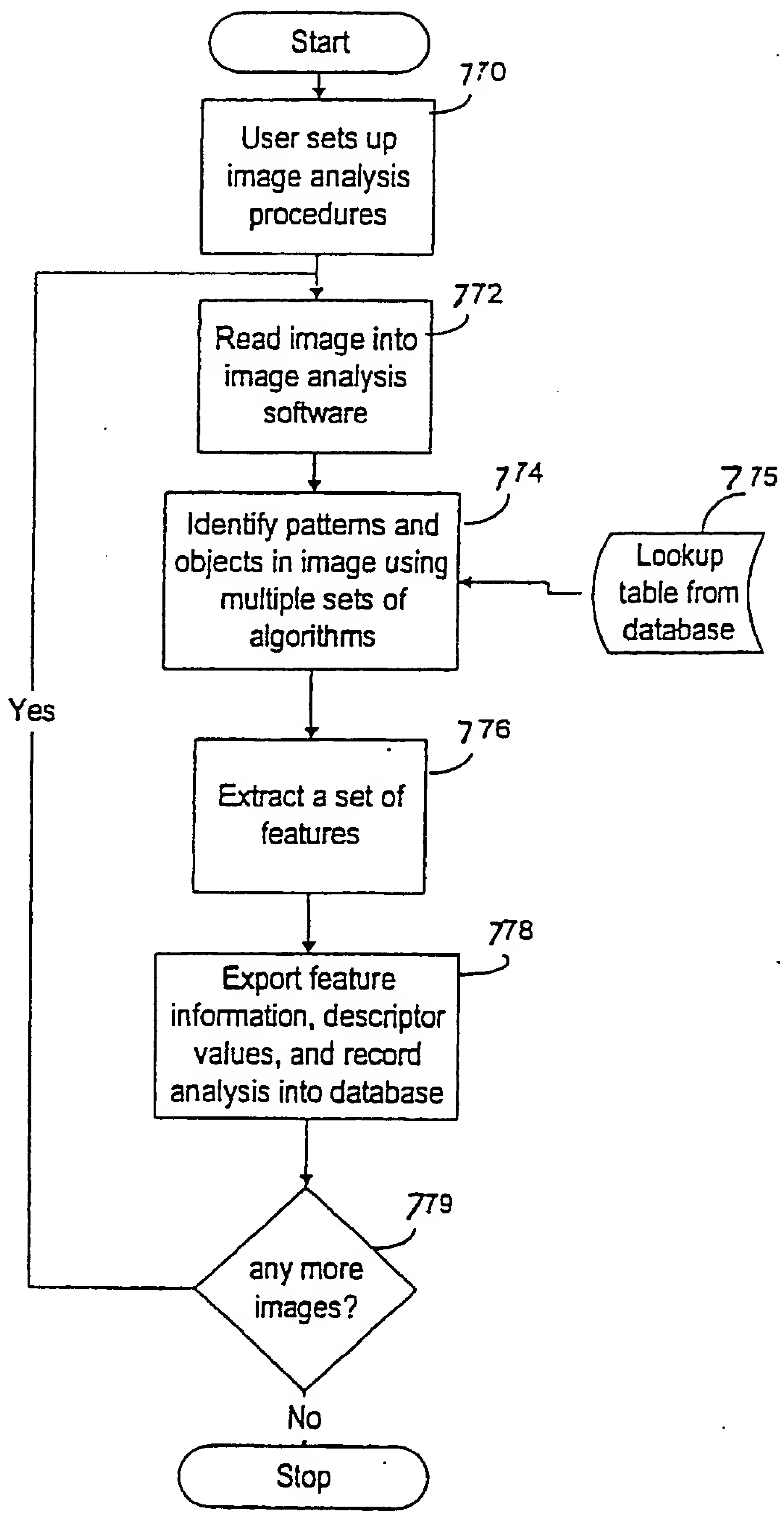

Start
770
User sets up image analysis procedures
772
Read image into image analysis software
774
775
Identify patterns and objects in image using multiple sets of algorithms
Lookup table from database
Yes
776
Extract a set of features
778
Export feature information, descriptor values, and record analysis into database
779
any more images?
No
Stop

Fig. 71

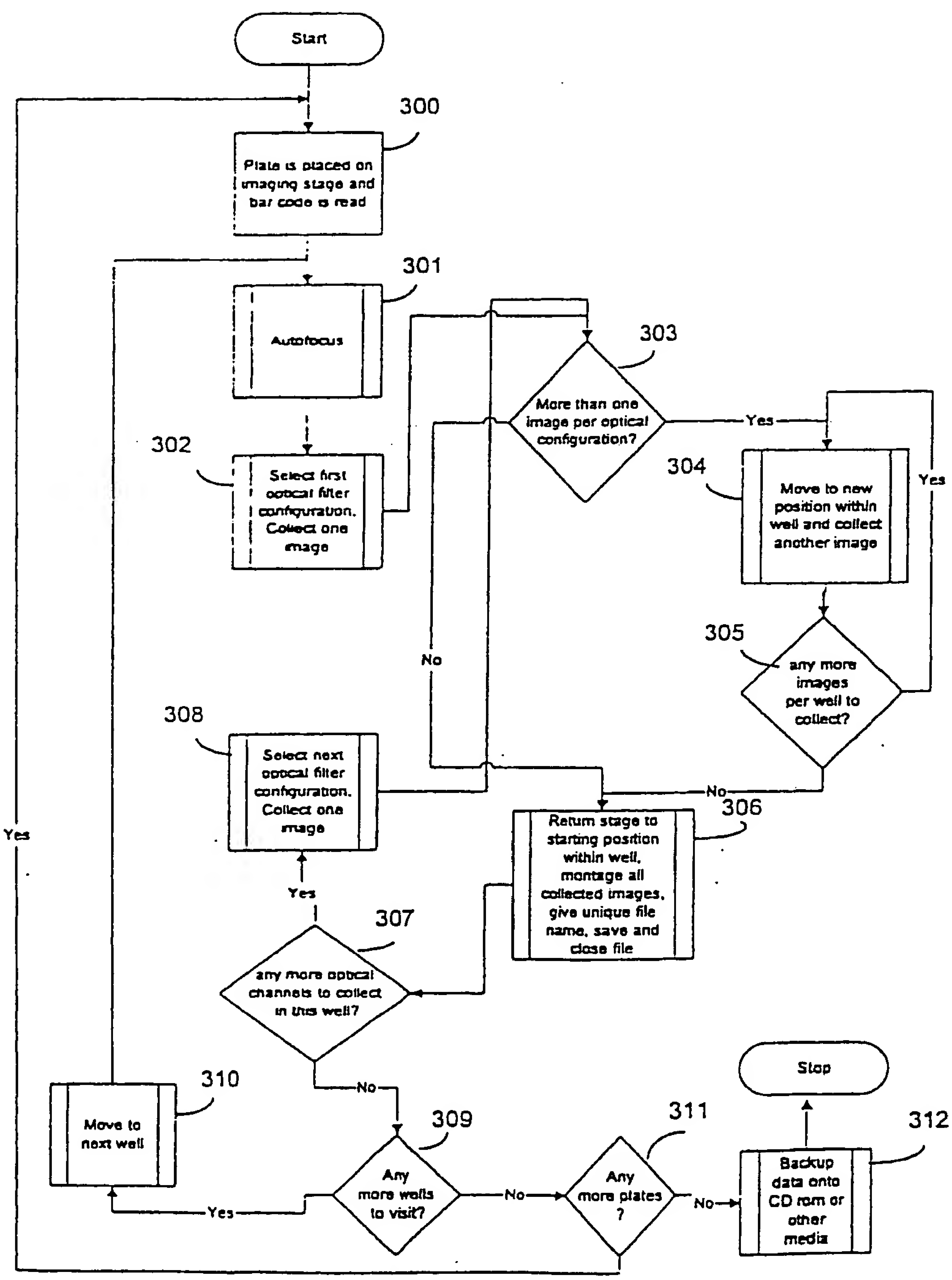

Start
300
Plate is placed on imaging stage and bar code is read
301
Autofocus
302
Select first optical filter configuration. Collect one image
303
More than one image per optical configuration?
Yes
304
Move to new position within well and collect another image
Yes
305
any more images per well to collect?
No
No
306
Return stage to starting position within well, montage all collected images, give unique file name, save and close file
308
Select next optical filter configuration. Collect one image
Yes
307
any more optical channels to collect in this well?
No
309
Any more wells to visit?
Yes
310
Move to next well
No
311
Any more plates ?
No
312
Backup data onto CD rom or other media
Stop
Yes

Fig. 7J

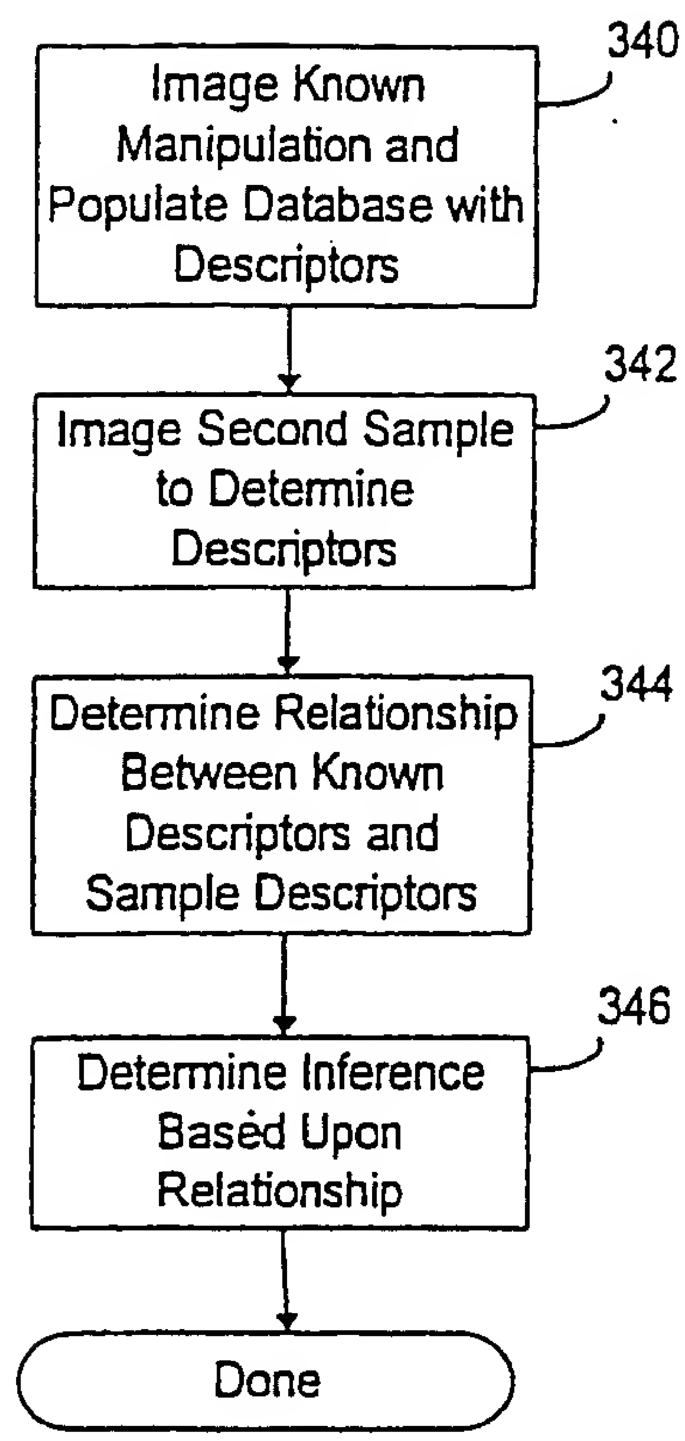

340
Image Known Manipulation and Populate Database with Descriptors
342
Image Second Sample to Determine Descriptors
344
Determine Relationship Between Known Descriptors and Sample Descriptors
346
Determine Inference Based Upon Relationship
Done

Fig. 7K

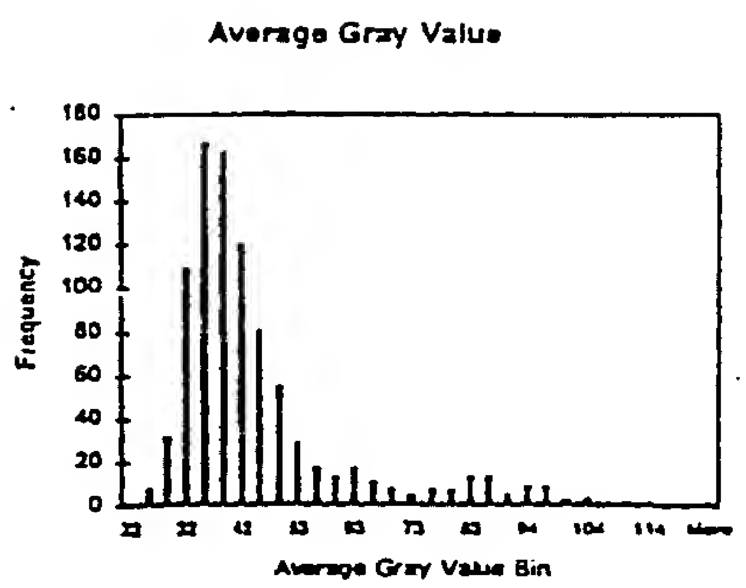


Fig. 8A

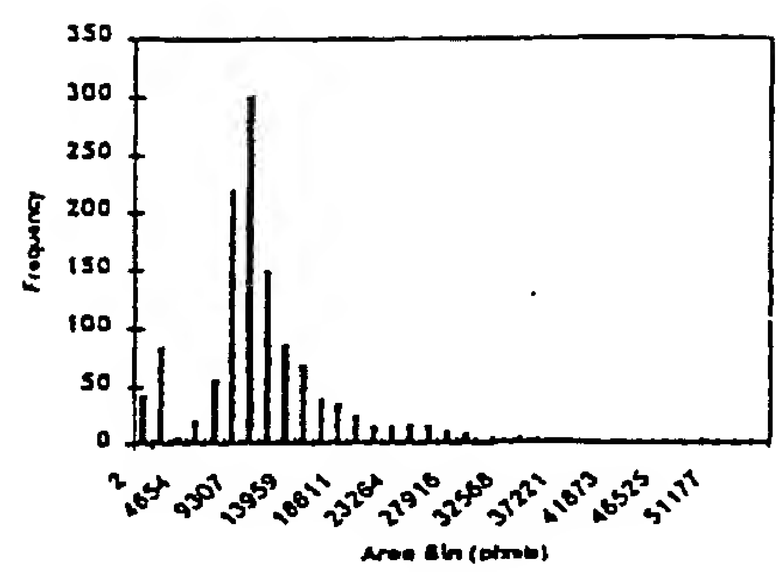


Fig. 8B

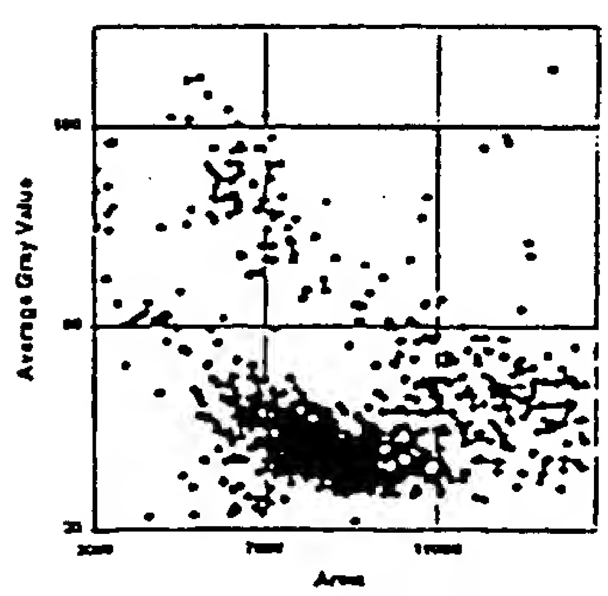


Fig. 8C

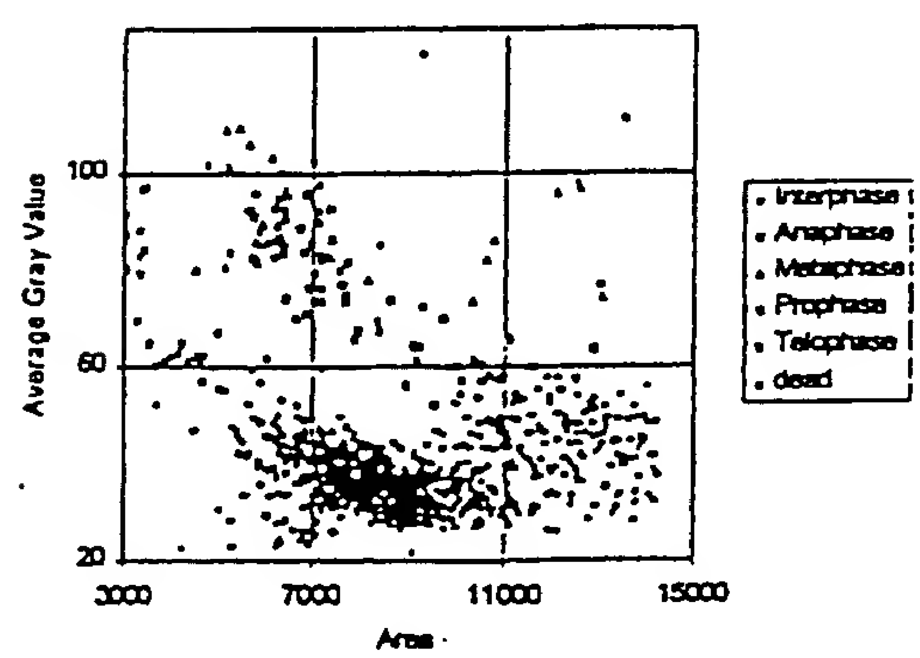


Fig. 8D

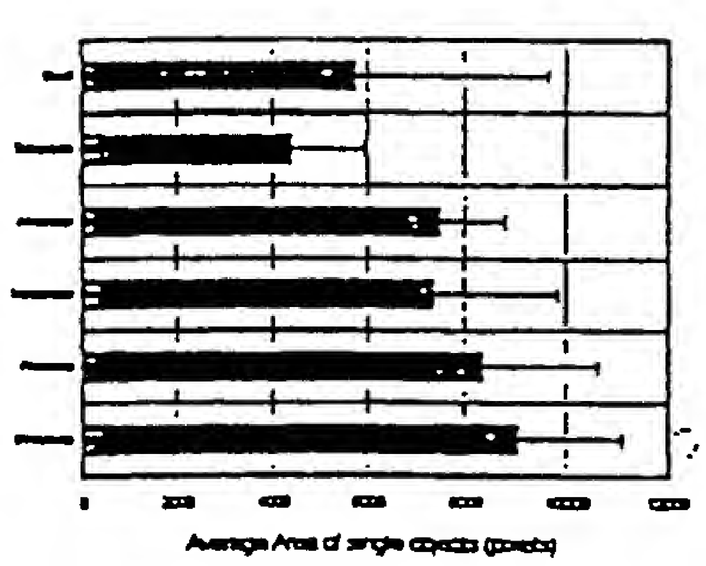


Fig. 8E

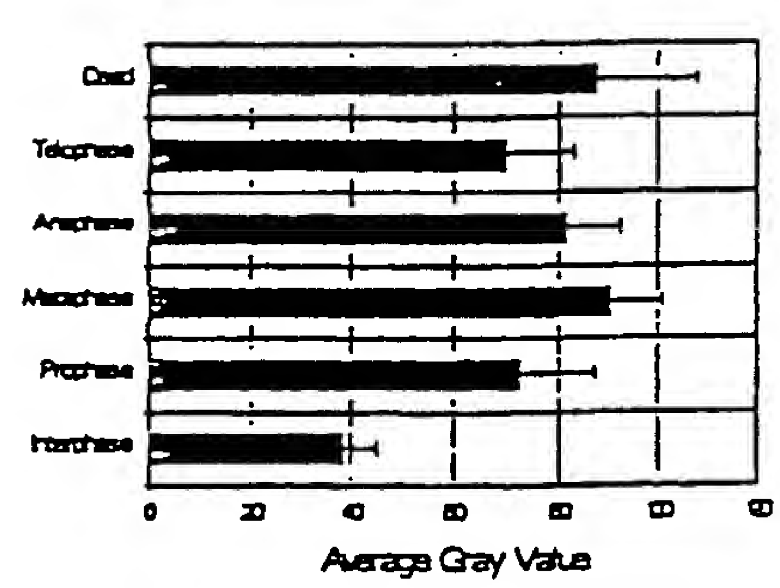
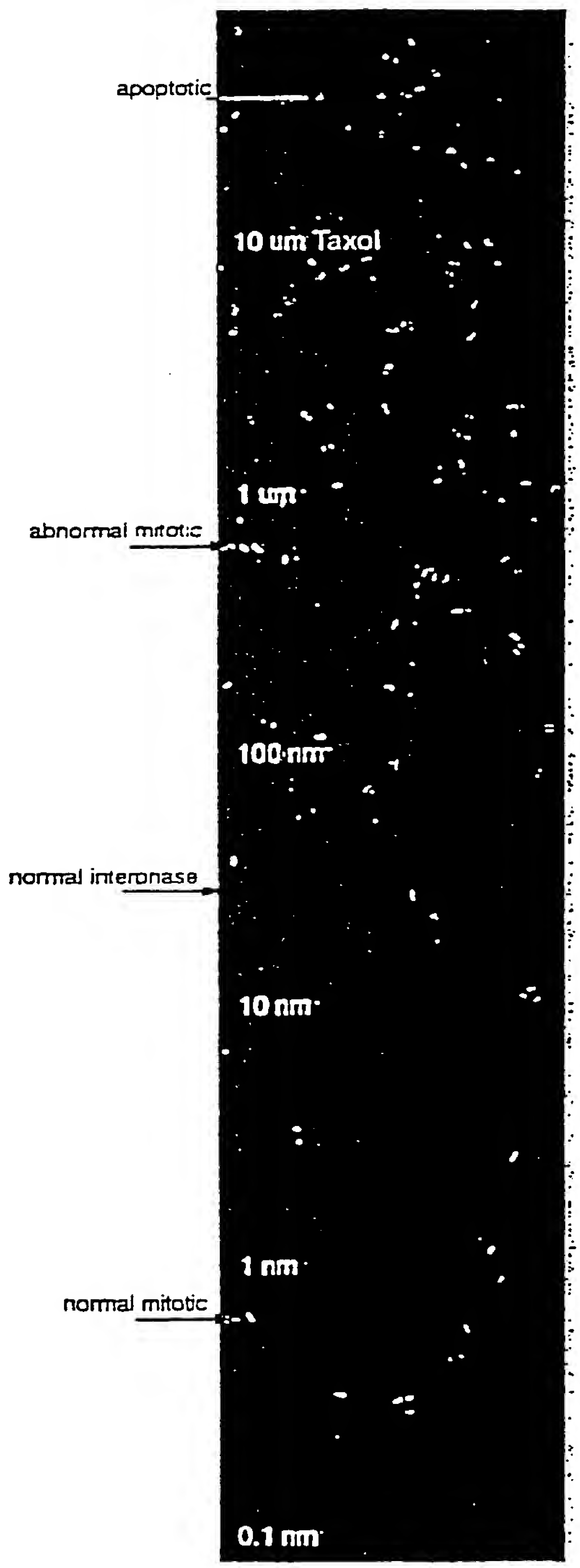


Fig. 8F



apoptotic
10 um Taxol
1 um
abnormal mitotic
100 nm
normal interphase
10 nm
1 nm
normal mitotic
0.1 nm

Fig.9

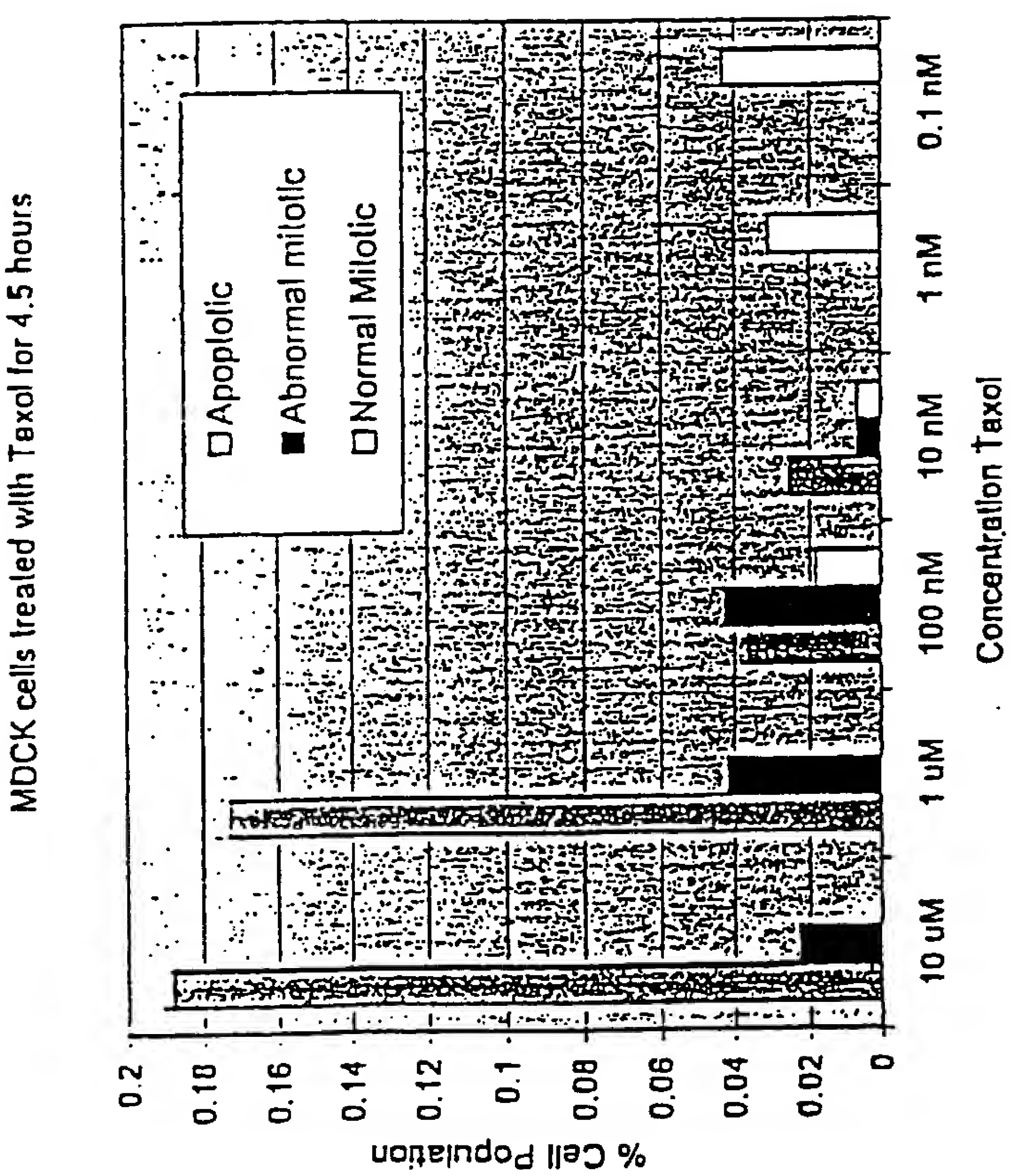

MDCK cells treated with Taxol for 4.5 hours
Apoptotic
Abnormal mitotic
Normal Mitotic
% Cell Population
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
10 uM
1 uM
100 nM
10 nM
1 nM
0.1 nM
Concentration Taxol

Fig. 10

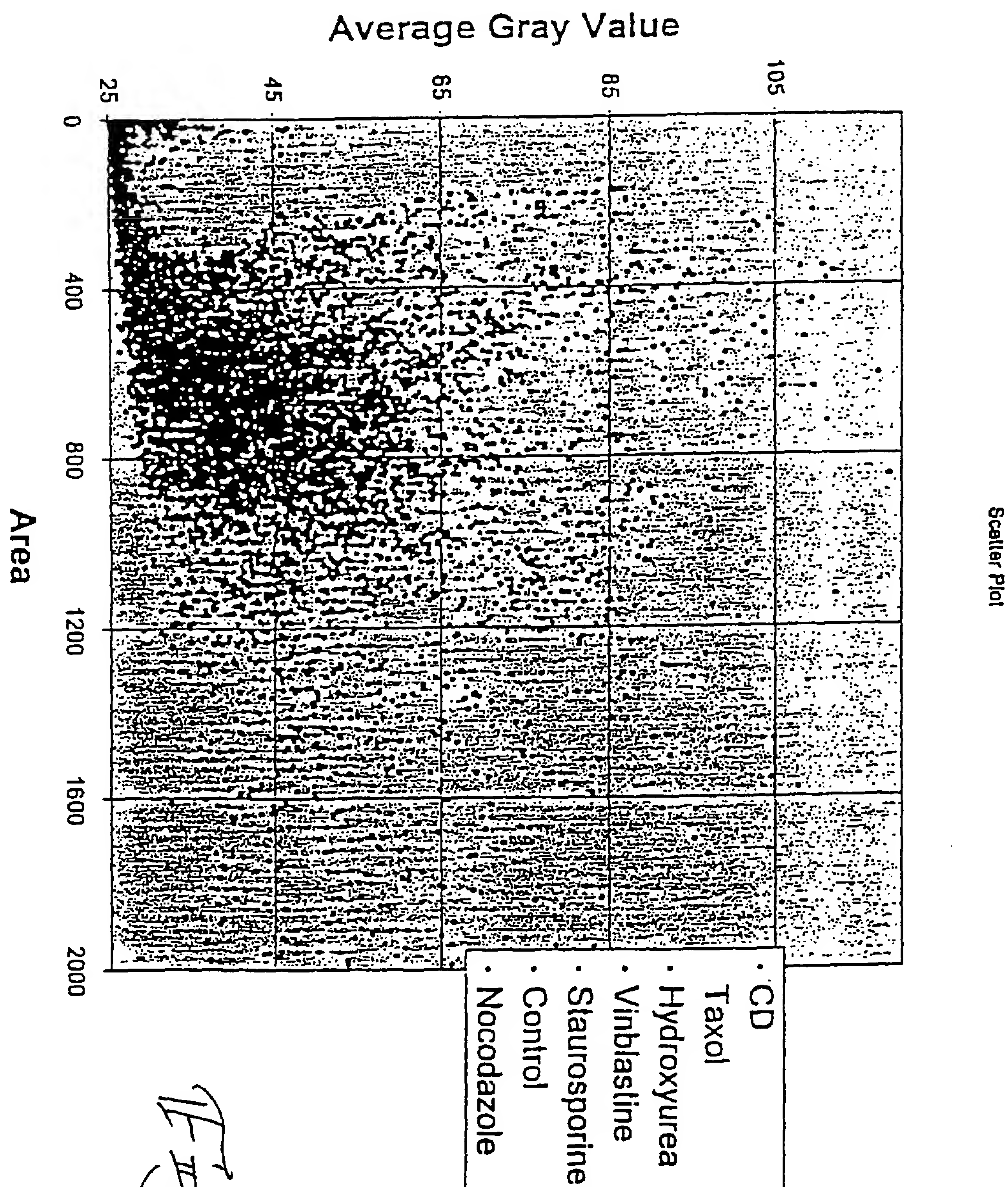

Average Gray Value
25
45
65
85
105
Area
0
400
800
1200
1600
2000
CD
Taxol
Hydroxyurea
Vinblastine
Staurosporine
Control
Nocodazole
Scatter Plot

Fig 11

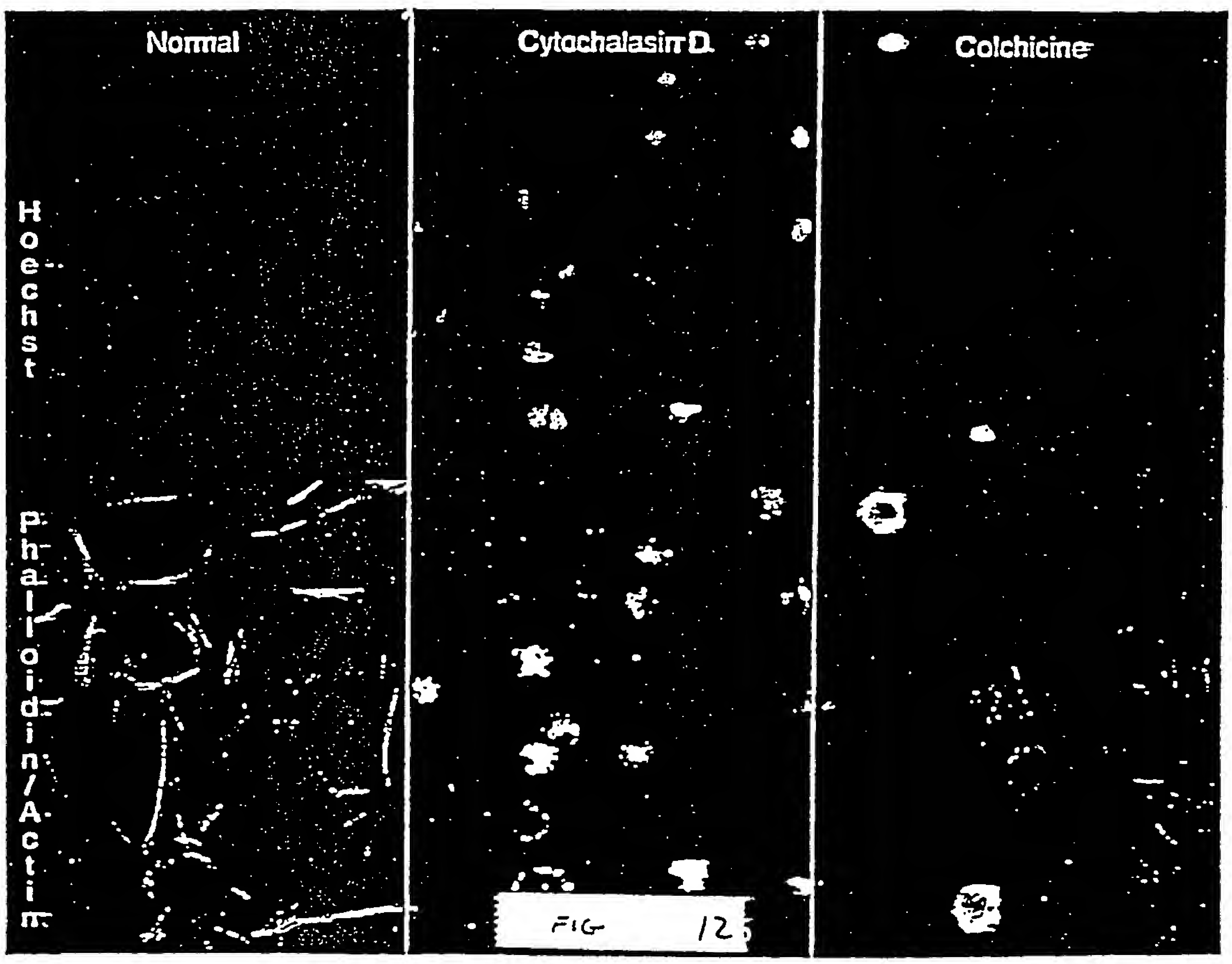

Normal
Cytochalasin D
Colchicine
Hoechst
Phalloidin/Actin

FIG 12

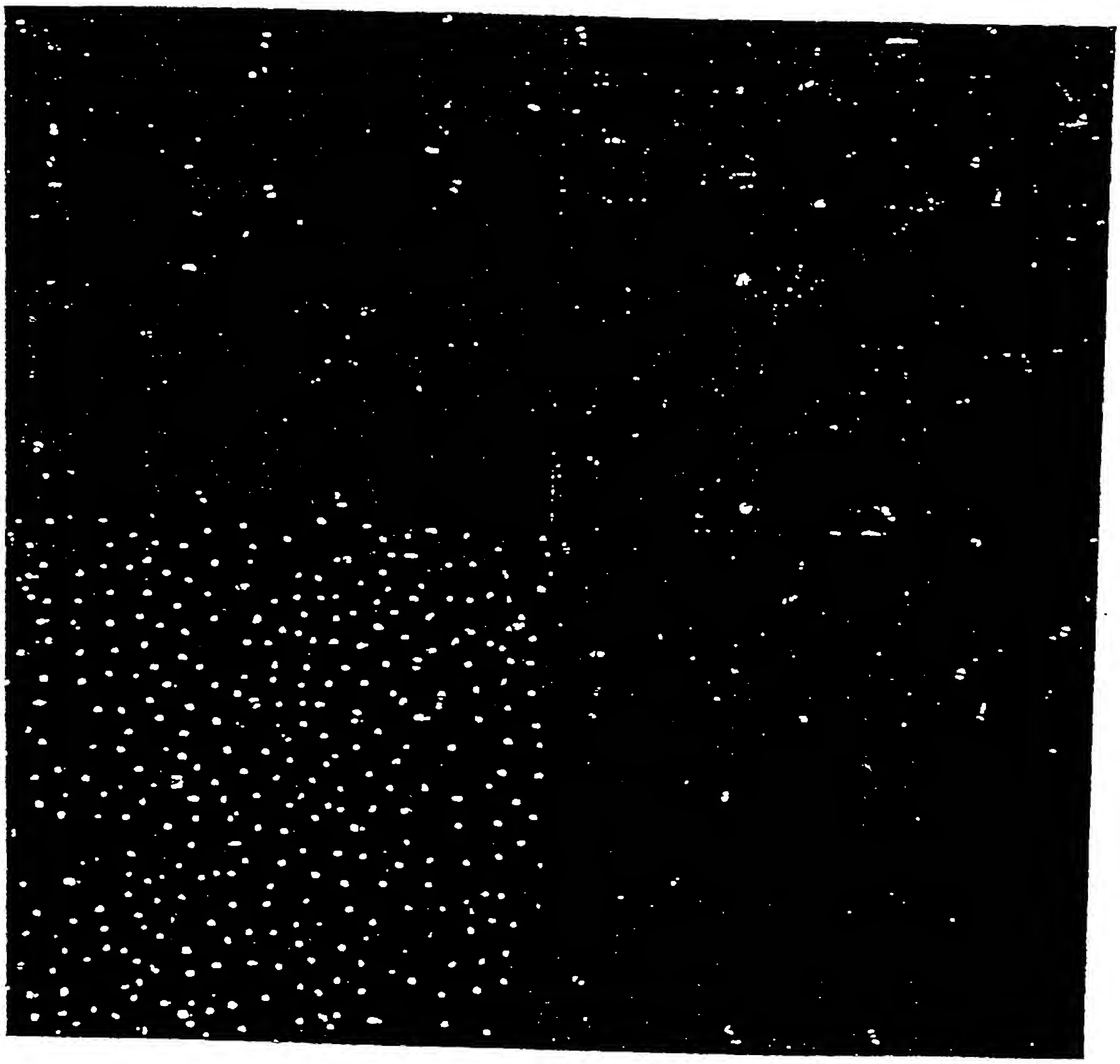


Fig 13

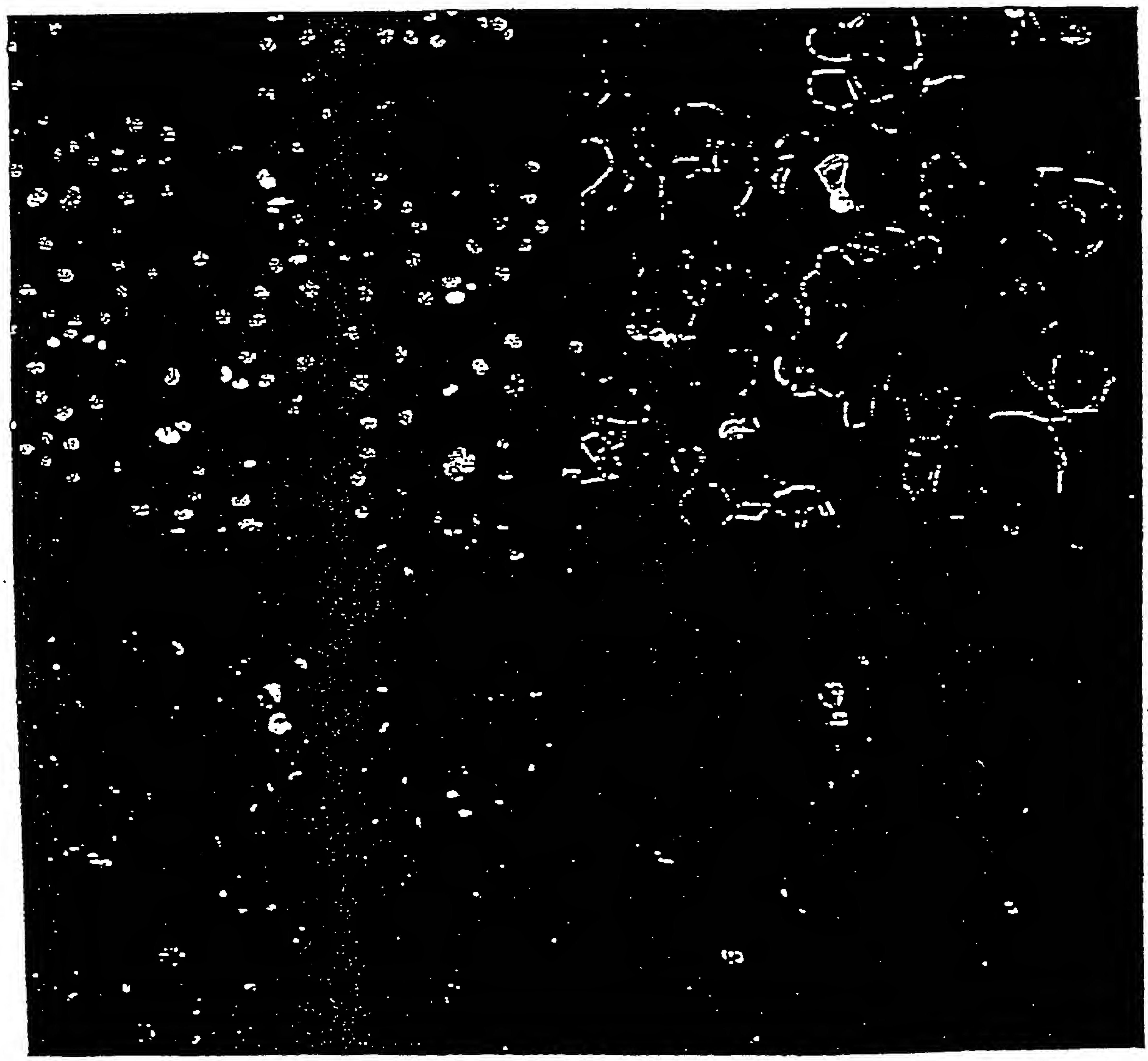


Fig 14

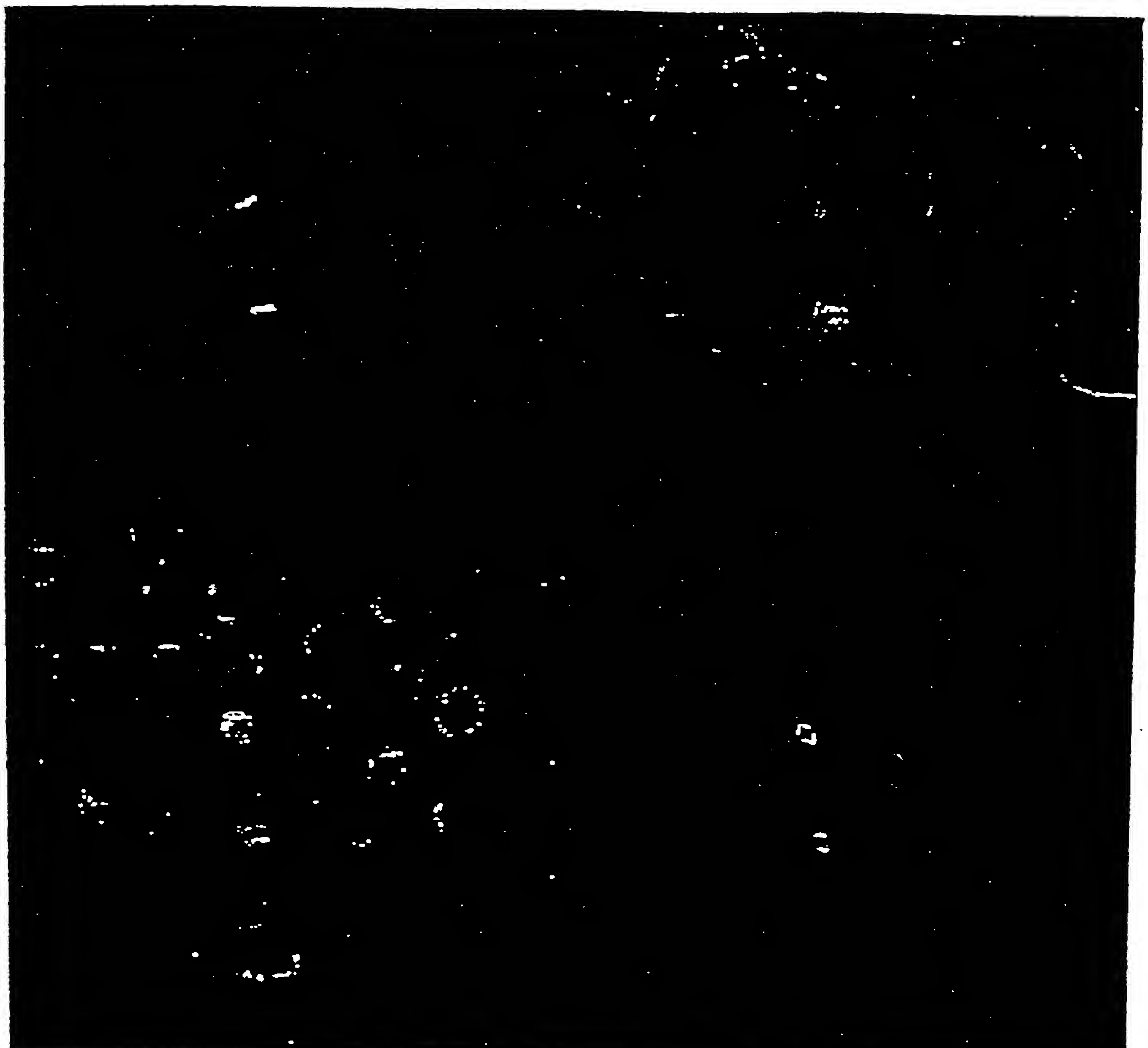


Fig 15

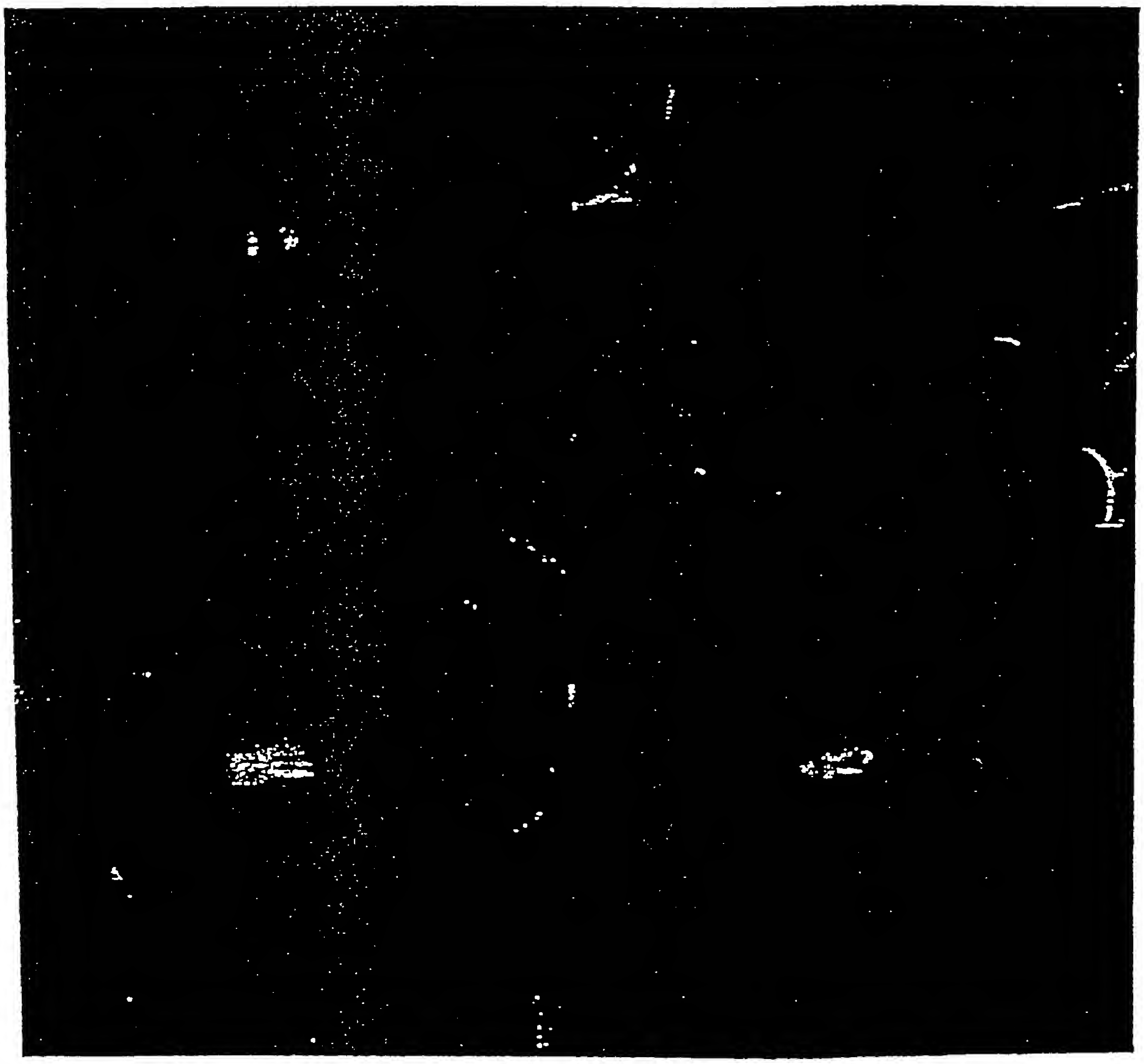


Fig 16

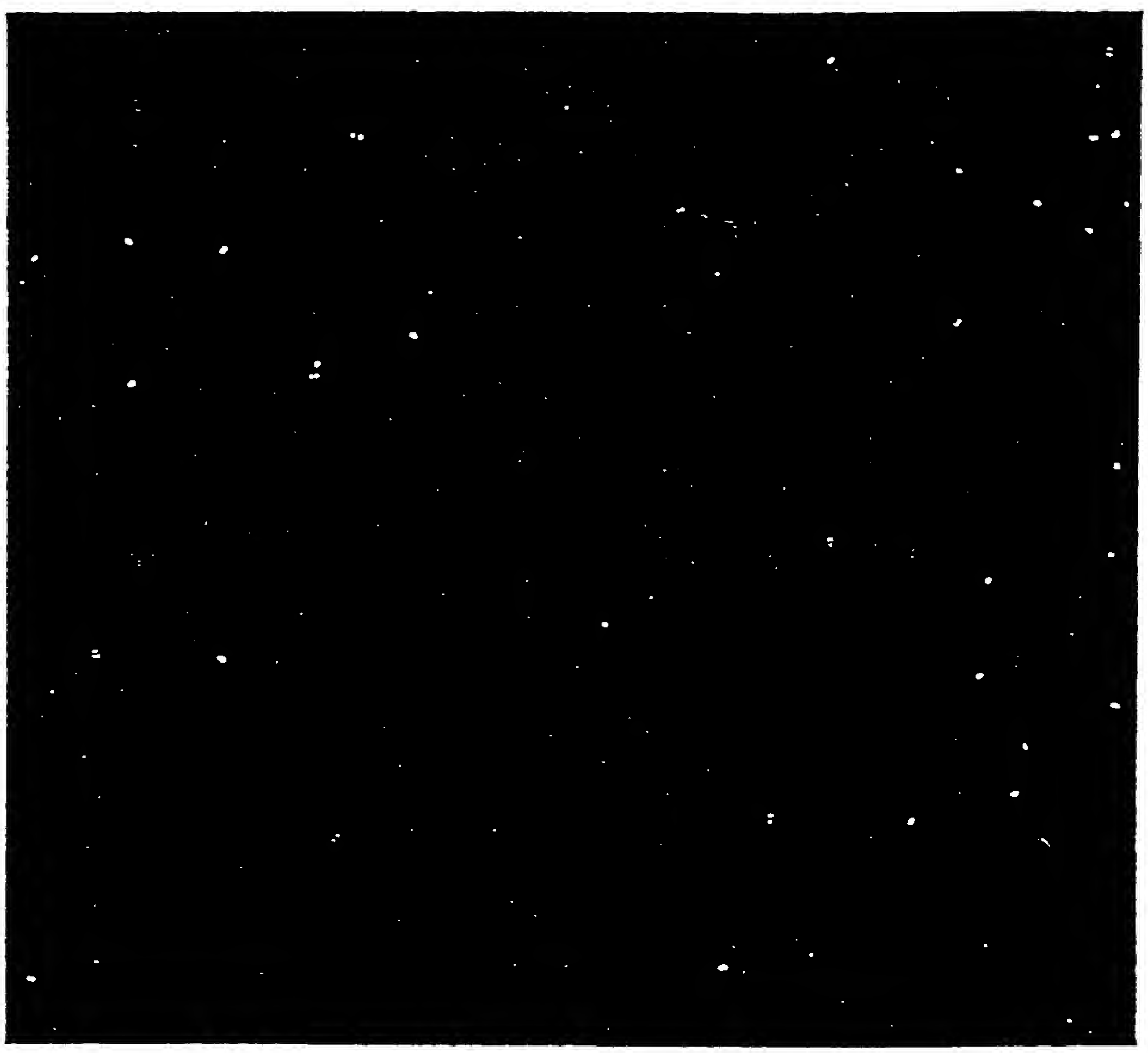


Fig 17

Conversion of morphometric parameters into nucleic acid code and clustering of the resulting sequences using Neighbor Joining method.

Compound:	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Maj	EFA Harmonic 2, Semi-Min
	Measurements																							
Control	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	a	t	t
Taxol	a	t	t	t	t	t	t	t	a	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
CD	c	a	a	a	t	a	t	t	c	a	a	a	a	a	a	a	a	t	a	a	a	t	a	g
Nocodozol	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Staurosponne	g	g	c	a	a	t	a	a	t	g	a	a	a	t	g	g	g	a	a	t	a	t	a	a
Vinblastine	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	g	t	t	t	t	t
Hydroxyurea	g	t	t	t	t	t	t	g	t	t	t	t	t	t	t	t	t	t	t	c	t	a	t	t

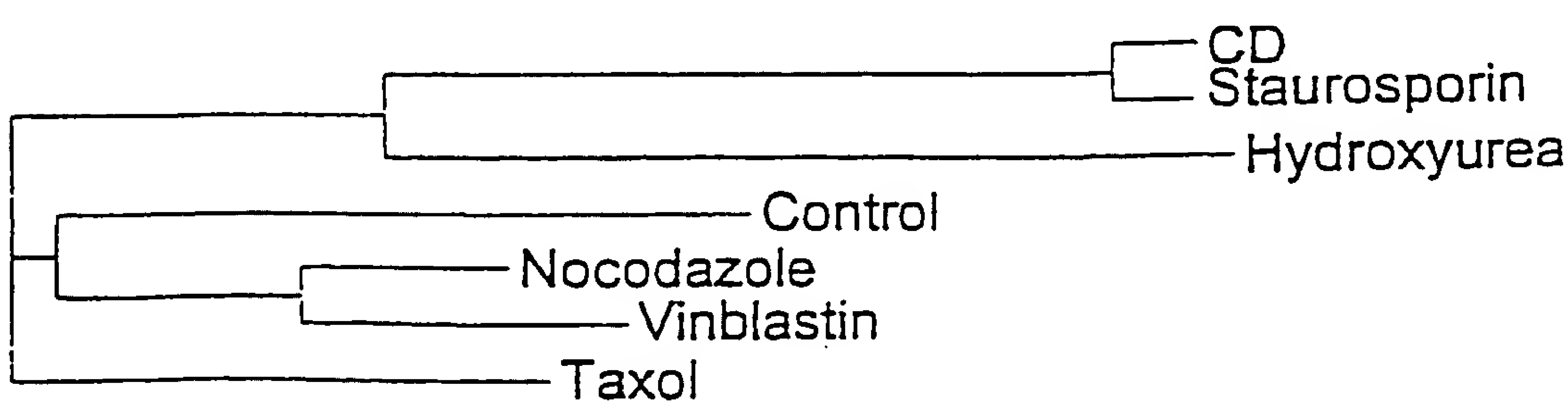


Fig 18

Conversion of morphometric parameters into amino acid codes and clustering of the resulting sequences using Neighbor Joining method.

	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface a	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Mo	EFA Harmonic 2, Semi-	EFA Harmonic 2, Semi-	
Control	H	P	T	T	N	S	D	W	E	S	T	T	T	F	C	C	P	P	M	C	T	G	T	T	Y
Taxol	G	F	M	M	P	M	P	H	G	S	M	M	W	C	F	P	F	R	C	M	M	H	M	P	S
CD	F	G	G	G	M	G	M	K	A	G	G	G	G	G	G	G	G	H	G	G	G	M	G	V	H
Nocodazol	W	F	M	M	W	M	P	T	R	S	M	M	M	F	M	W	F	M	M	R	M	M	M	F	G
Staurosporine	N	V	A	G	G	M	G	G	Y	V	G	G	G	M	V	V	V	G	G	H	G	M	G	G	V
Vinblastine	F	W	W	M	W	W	C	W	D	S	M	W	W	M	M	M	W	M	V	E	M	M	M	F	P
Hydroxyurea	S	H	H	H	H	H	H	V	H	H	H	H	H	H	H	H	H	H	H	A	H	G	H	H	D

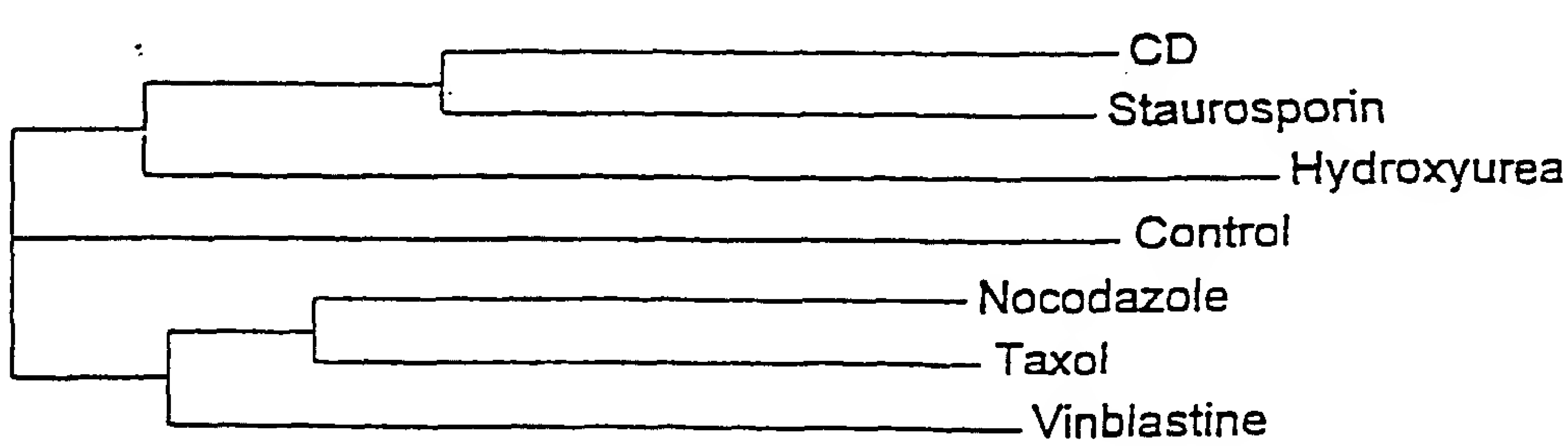
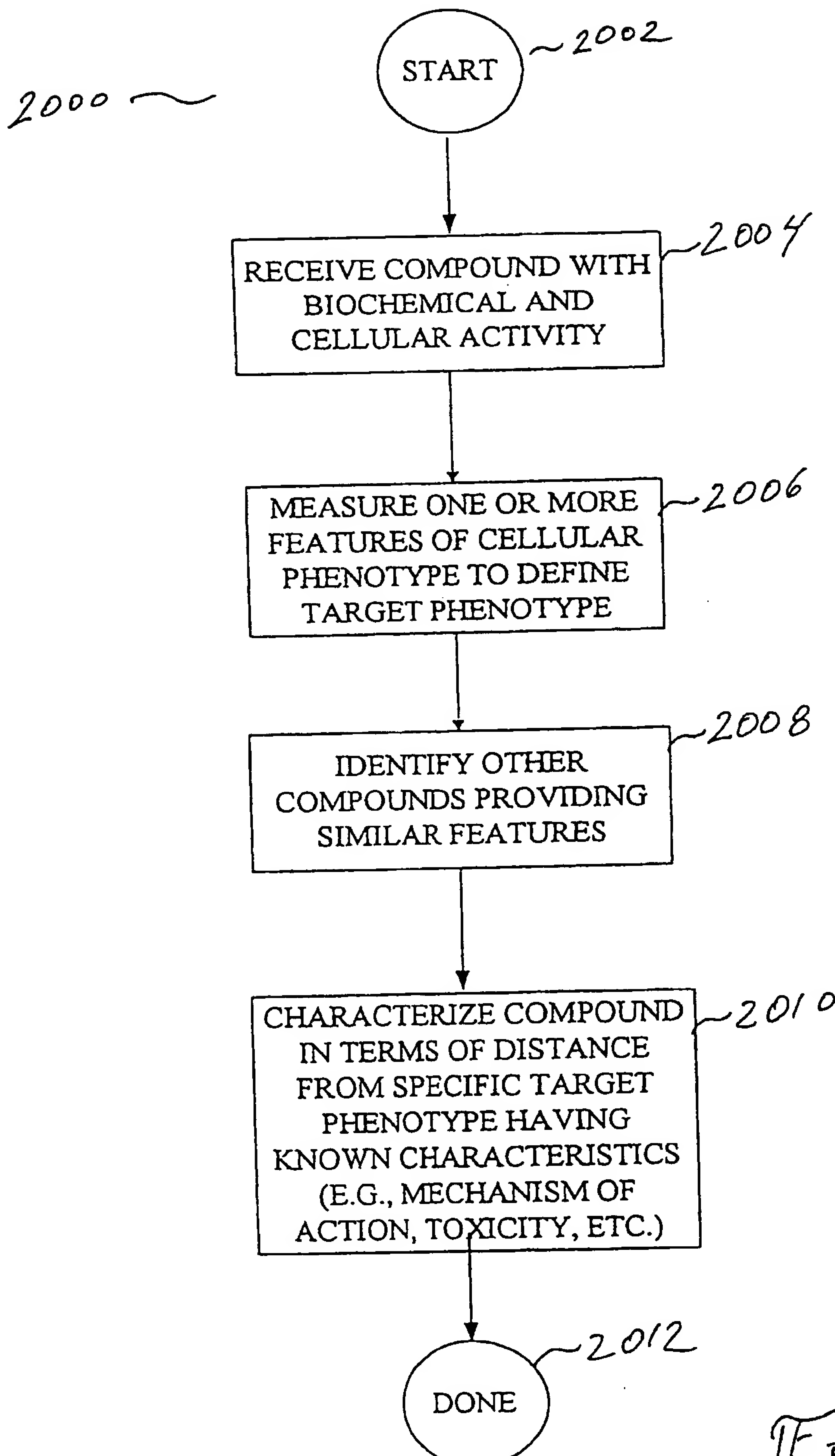


Fig 19



2000
START
2002
RECEIVE COMPOUND WITH BIOCHEMICAL AND CELLULAR ACTIVITY
2004
MEASURE ONE OR MORE FEATURES OF CELLULAR PHENOTYPE TO DEFINE TARGET PHENOTYPE
2006
IDENTIFY OTHER COMPOUNDS PROVIDING SIMILAR FEATURES
2008
CHARACTERIZE COMPOUND IN TERMS OF DISTANCE FROM SPECIFIC TARGET PHENOTYPE HAVING KNOWN CHARACTERISTICS (E.G., MECHANISM OF ACTION, TOXICITY, ETC.)
2010
DONE
2012

Fig 20

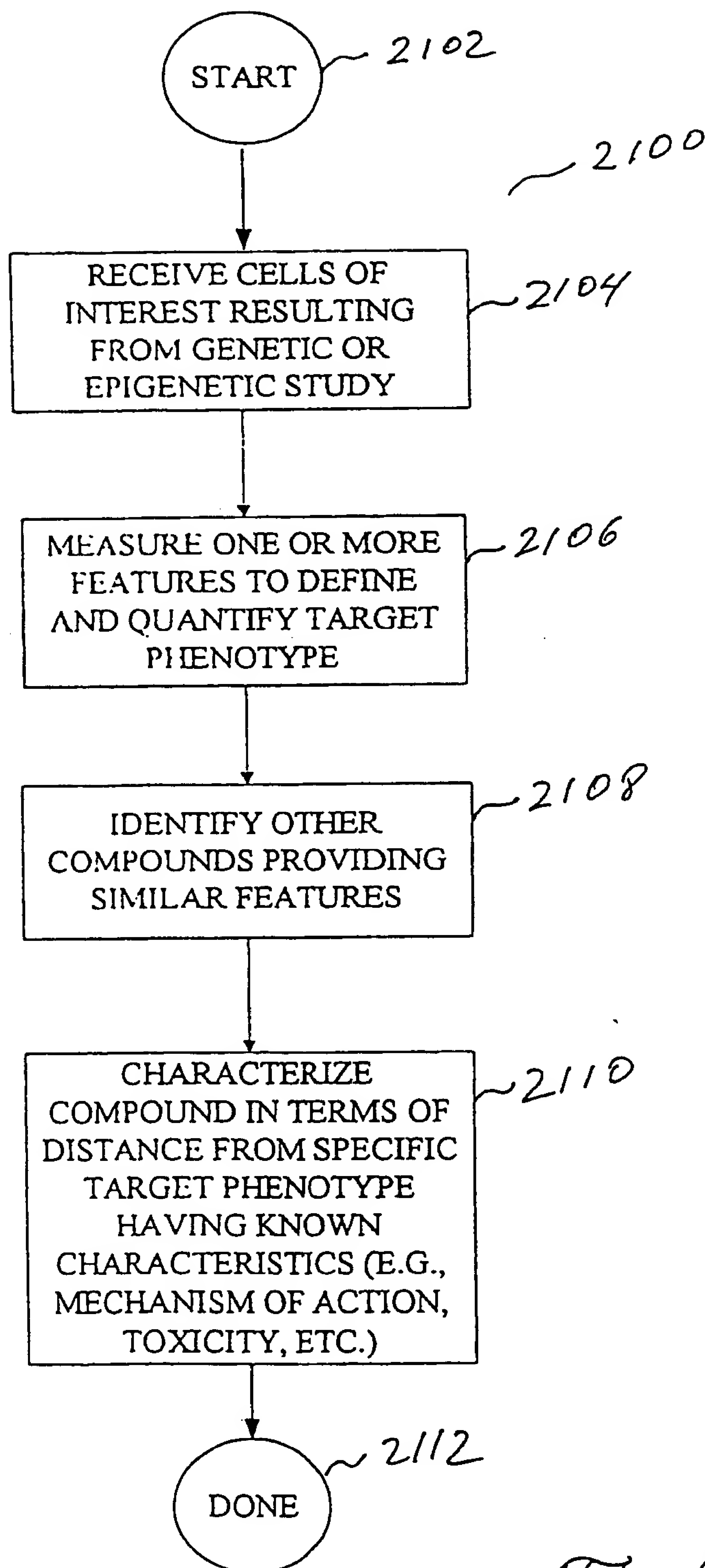

START
2102
2100
RECEIVE CELLS OF INTEREST RESULTING FROM GENETIC OR EPIGENETIC STUDY
2104
MEASURE ONE OR MORE FEATURES TO DEFINE AND QUANTIFY TARGET PHENOTYPE
2106
IDENTIFY OTHER COMPOUNDS PROVIDING SIMILAR FEATURES
2108
CHARACTERIZE COMPOUND IN TERMS OF DISTANCE FROM SPECIFIC TARGET PHENOTYPE HAVING KNOWN CHARACTERISTICS (E.G., MECHANISM OF ACTION, TOXICITY, ETC.)
2110
DONE
2112

Fig 21

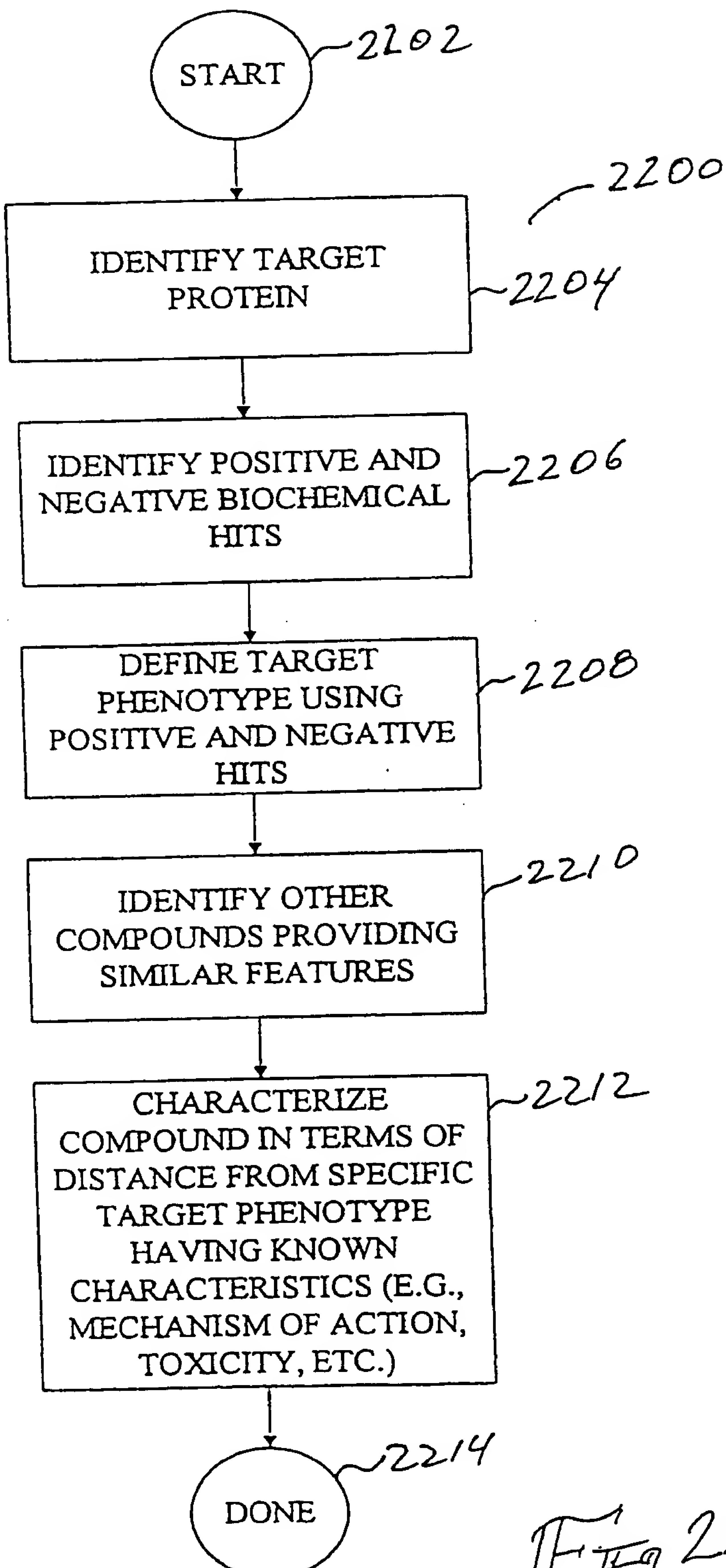

START
2202
2200
IDENTIFY TARGET PROTEIN
2204
IDENTIFY POSITIVE AND NEGATIVE BIOCHEMICAL HITS
2206
DEFINE TARGET PHENOTYPE USING POSITIVE AND NEGATIVE HITS
2208
IDENTIFY OTHER COMPOUNDS PROVIDING SIMILAR FEATURES
2210
CHARACTERIZE COMPOUND IN TERMS OF DISTANCE FROM SPECIFIC TARGET PHENOTYPE HAVING KNOWN CHARACTERISTICS (E.G., MECHANISM OF ACTION, TOXICITY, ETC.)
2212
DONE
2214

Fig 22